

Title: NUCLEIC ACID AND CORRESPONDING PROTEIN
ENTITLED 101P3A41 USEFUL IN TREATMENT AND
DETECTION OF CANCER

First Inventor: Daniel E. H. AFAR, et al

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Application No.: To be Assigned - Docket No. 511582002420

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Figure 1

GATCAAACCTTTCCATTGAGTCCTCTGATTGAGATTTAACATTGGAAAGACAGTATTGAGAAAAAAATTCC
TTAATAAAAATACAACCTGAGCTTCAAAATATGAAACTGGTGGGAAATCTCCATTTCATATTATTCTCTTGTTC
TTGCTACGTATAATTATAATCTGACTAGGTTGTGGTGGAGGGTTATTACTTTCATTTACATGCAGTCCAAATCTAAAC
TGCTTCACTGATGGTTACAGCATTCTGAGATAAGAATGGTACATCTAGAGAACATTGCCAAGGCCAAGCAGCAAAGGAA
AATAAACACAGAAATAATAAAATGATAATCTAGCTAAAACATTAACCTCCCTTTAGAATCTCCAAACACATTGGATC

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FIG. 2A

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9	18	27	36	45	54
5' CAG AGA GGC TGT ATT TCA GTG CAG CCT GCC AGA CCT CTT CTG GAG GAA GAC TGG					

63	72	81	90	99	108
ACA AAG GGG GTC ACA CAT TCC TTC CAT ACG GTT GAG CCT CTA CCT GCC TGG TGC					

117	126	135	144	153	162
TGG TCA CAG TTC AGC TTC <u>ATG</u> ATG GTG GAT CCC AAT GGC AAT GAA TCC AGT					

M	M	V	D	P	N
G	G	L	P	G	N
N	N	E	D	S	S

171	180	189	198	207	216
GCT ACA TAC TTC ATC CTA ATA GGC CTC CCT GGT TTA GAA GAG GCT CAG TTC TGG					

A	T	Y	F	I	L
I	G	L	P	G	L
E	E	S	A	Q	F
S	A	W			

225	234	243	252	261	270
TTG GCC TTC CCA TTG TGC TCC CTC TAC CTT ATT GCT GTG CTA GGT AAC TTG ACA					

L	A	F	P	L	C
S	S	Y	L	I	G
L	V	I	A	V	N
T	R	T	I	L	L

I	I	Y	I	E	E
I	V	R	H	S	L
F	T	S	H	E	P
R	E	P	E	M	Y

279	288	297	306	315	324
ATC ATC TAC ATT GTG CGG ACT GAG CAC AGC CTG CAT GAG CCC ATG TAT ATA TTT					

I	I	Y	I	F	F
I	V	R	T	E	W
R	E	S	H	S	H

333	342	351	360	369	378
CTT TGC ATG CTT TCA GGC ATT GAC ATC CTC ATC TCC ACC TCA TCC ATG CCC AAA					

L	C	M	L	S	G
S	I	D	I	L	I
G	D	T	I	S	T
T	T	T	I	S	S

387	396	405	414	423	432
ATG CTG GCC ATC TTC TGG TTC AAT TCC ACT ACC ATC CAG TTT GAT GCT TGT CTG					

M	L	A	I	F	W
L	N	S	T	T	N
A	S	S	T	I	E
T	T	T	I	P	K

441	450	459	468	477	486
CTA CAG ATT TTT GCC ATC CAC TCC TTA TCT GGC ATG GAA TCC ACA GTG CTG CTG					

L	Q	I	F	A	I
Q	H	S	L	S	G
I	H	S	I	M	E
F	S	T	G	E	S
A	T	S	I	T	V

495	504	513	522	531	540
GCC ATG GCT TTT GAC CGC TAT GTG GCC ATC TGT CAC CCA CTG CGC CAT GCC ACA					

A	M	A	F	D	R
M	A	Y	V	I	H
A	V	A	I	C	P
T	I	C	H	H	L

549	558	567	576	585	594
GTA CTT ACG TTG CCT CGT GTC ACC AAA ATT GGT GTG GCT GCT GTG GTG CGG GGG					

V	L	T	P	R	G
L	V	R	V	V	G
T	T	K	I	A	A
P	K	G	V	V	R

603	612	621	630	639	648
GCT GCA CTG ATG GCA CCC CCT GTC TTC ATC AAG CAG CTG CCC TTC TGC CGC					

A	A	L	M	A	P
A	M	A	P	L	P
L	P	V	F	I	K
P	V	F	I	Q	L
V	F	I	K	Q	P
F	S	T	P	F	C
R	G	I	C	R	

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FIG. 2B

657	666	675	684	693	702
TCC AAT ATC CTT TCC CAT TCC TAC TGC CTA CAC CAA GAT GTC ATG AAG CTG GCC					
S N I L S H S Y C L H Q D V M K L A					
711	720	729	738	747	756
TGT GAT GAT ATC CGG GTC AAT GTC GTC TAT GGC CTT ATC GTC ATC ATC TCC GCC					
C D D I R V N V V Y G L I V I I S A					
765	774	783	792	801	810
ATT GGC CTG GAC TCA CTT CTC ATC TCC TTC TCA TAT CTG CTT ATT CTT AAG ACT					
I G L D S L L I S F S Y L L I L K T					
819	828	837	846	855	864
GTG TTG GGC TTG ACA CGT GAA GCC CAG GCC AAG GCA TTT GGC ACT TGC GTC TCT					
V L G L T R E A Q A K A F G T C V S					
873	882	891	900	909	918
CAT GTG TGT GCT GTG TTC ATA TTC TAT GTA CCT TTC ATT GGA TTG TCC ATG GTG					
H V C A V F I F Y V P F I G L S M V					
927	936	945	954	963	972
CAT CGC TTT AGC AAG CGG CGT GAC TCT CCG CTG CCC GTC ATC TTG GCC AAT ATC					
H R F S K R R D S P L P V I L A N I					
981	990	999	1008	1017	1026
TAT CTG CTG GTT CCT CCT GTG CTC AAC CCA ATT GTC TAT GGA GTG AAG ACA AAG					
Y L L V P P V L N P I V Y G V K T K					
1035	1044	1053	1062	1071	1080
GAG ATT CGA CAG CGC ATC CTT CGA CTT TTC CAT GTG GCC ACA CAC GCT TCA GAG					
E I R Q R I L R L F H V A T H A S E					
1089	1098	1107	1116	1125	1134
CCC TAG GTG TCA GTG ATC AAA CTT CTT TTC CAT TCA GAG TCC TCT GAT TCA GAT					
P *					
1143	1152	1161	1170	1179	1188
TTT AAT GTT AAC ATT TTG GAA GAC AGT ATT CAG AAA AAA AAT TTC CTT AAT AAA					
1197	1206	1215	1224	1233	1242
AAA TAC AAC TCA GAT CCT TCA AAT ATG AAA CTG GTT GGG GAA TCT CCA TTT TTT					
1251	1260	1269	1278	1287	1296
CAA TAT TAT TTT CTT CTT TGT TTT CTT GCT ACA TAT AAT TAT TAA TAC CCT GAC					
1305	1314	1323	1332	1341	1350
TAG GTT GTG GTT GGA GGG TTA TTA CTT TTC ATT TTA CCA TGC AGT CCA AAT CTA					

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FIG. 2C

1359	1368	1377	1386	1395	1404
AAC TGC TTC TAC TGA TGG TTT ACA GCA TTC TGA GAT AAG AAT GGT ACA TCT AGA					
-----	-----	-----	-----	-----	-----
1413	1422	1431	1440	1449	1458
GAA CAT TTG CCA AAG GCC TAA GCA CGG CAA AGG AAA ATA AAC ACA GAA TAT AAT					
-----	-----	-----	-----	-----	-----
1467	1476	1485	1494	1503	1512
AAA ATG AGA TAA TCT AGC TTA AAA CTA TAA CCT CCT CTT CAG AAC TCC CAA CCA					
-----	-----	-----	-----	-----	-----
1521	1530	1539	1548	1557	1566
CAT TGG ATC TCA GAA AAA TGC TGT CTT CAA AAT GAC TTC TAC AGA GAA GAA ATA					
-----	-----	-----	-----	-----	-----
1575	1584	1593	1602	1611	1620
ATT TTT CCT CTG GAC ACT AGC ACT TAA GGG GAA GAT TGG AAG TAA AGC CTT GAA					
-----	-----	-----	-----	-----	-----
1629	1638	1647	1656	1665	1674
AAG AGT ACA TTT ACC TAC GTT AAT GAA AGT TGA CAC ACT GTT CTG AGA GTT TTC					
-----	-----	-----	-----	-----	-----
1683	1692	1701	1710	1719	1728
ACA GCA TAT GGA CCC TGT TTT TCC TAT TTA ATT TTC TTA TCA ACC CTT TAA TTA					
-----	-----	-----	-----	-----	-----
1737	1746	1755	1764	1773	1782
GGC AAA GAT ATT ATT AGT ACC CTC ATT GTA GCC ATG GGA AAA TTG ATG TTC AGT					
-----	-----	-----	-----	-----	-----
1791	1800	1809	1818	1827	1836
GGG GAT CAG TGA ATT AAA TGG GGT CAT ACA AGT ATA AAA ATT AAA AAA AAA AAA					
-----	-----	-----	-----	-----	-----
1845	1854	1863	1872	1881	1890
GAC TTC ATG CCC AAT CTC ATA TGA TGT GGA AGA ACT GTT AGA GAG ACC AAC AGG					
-----	-----	-----	-----	-----	-----
1899	1908	1917	1926	1935	1944
GTA GTG GGT TAG AGA TTT CCA GAG TCT TAC ATT TTC TAG AGG AGG TAT TTA ATT					
-----	-----	-----	-----	-----	-----
1953	1962	1971	1980	1989	1998
TCT TCT CAC TCA TCC AGT GTT GTA TTT AGG AAT TTC CTG GCA ACA GAA CTC ATG					
-----	-----	-----	-----	-----	-----
2007	2016	2025	2034	2043	2052
GCT TTA ATC CCA CTA GCT ATT GCT TAT TGT CCT GGT CCA ATT GCC AAT TAC CTG					
-----	-----	-----	-----	-----	-----
2061	2070	2079	2088	2097	2106
TGT CTT GGA AGA AGT GAT TTC TAG GTT CAC CAT TAT GGA AGA TTC TTA TTC AGA					
-----	-----	-----	-----	-----	-----
2115	2124	2133	2142	2151	2160
AAG TCT GCA TAG GGC TTA TAG CAA GTT ATT TAT TTT TAA AAG TTC CAT AGG TGA					
-----	-----	-----	-----	-----	-----
2169	2178	2187	2196	2205	2214
TTC TGA TAG GCA GTG AGG TTA GGG AGC CAC CAG TTA TGA TGG GAA GTA TGG AAT					
-----	-----	-----	-----	-----	-----
2223	2232	2241	2250	2259	2268
GGC AGG TCT TGA AGA TAA CAT TGG CCT TTT GAG TGT GAC TCG TAG CTG GAA AGT					
-----	-----	-----	-----	-----	-----
2277	2286	2295	2304	2313	2322
GAG GGA ATC TTC AGG ACC ATG CTT TAT TTG GGG CTT TGT GCA GTA TGG AAC AGG					
-----	-----	-----	-----	-----	-----
2331	2340	2349	2358	2367	2376
GAC TTT GAG ACC AGG AAA GCA ATC TGA CTT AGG CAT GGG AAT CAG GCA TTT TTG					
-----	-----	-----	-----	-----	-----

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FIG. 2D

2385	2394	2403	2412	2421	2430
CTT CTG AGG GGC TAT TAC CAA GGG TTA ATA GGT TTC ATC TTC AAC AGG ATA TGA					

2439	2448	2457	2466	2475	2484
CAA CAG TGT TAA CCA AGA AAC TCA AAT TAC AAA TAC TAA AAC ATG TGA TCA TAT					

2493	2502	2511	2520	2529	2538
ATG TGG TAA GTT TCA TTT TCT TTT TCA ATC CTC AGG TTC CCT GAT ATG GAT TCC					

2547	2556	2565	2574	2583	2592
TAT AAC ATG CTT TCA TCC CCT TTT GTA ATG GAT ATC ATA TTT GGA AAT GCC TAT					

2601	2610	2619	2628	2637	2646
TTA ATA CTT GTA TTT GCT GCT GGA CTG TAA GCC CAT GAG GGC ACT GTT TAT TAT					

2655	2664	2673	2682	2691	2700
TGA ATG TCA TCT CTG TTC ATC ATT GAC TGC TCT TTG CTC ATC ATT GAA TCC CCC					

2709	2718	2727	2736	2745	2754
AGC AAA GTG CCT AGA ACA TAA TAG TGC TTA TGC TTG ACA CCG GTT ATT TTT CAT					

2763	2772	2781	2790	2799	2808
CAA ACC TGA TTC CTT CTG TCC TGA ACA CAT AGC CAG GCA ATT TTC CAG CCT TCT					

2817	2826	2835	2844	2853	2862
TTG AGT TGG GTA TTA TTA AAT TCT GGC CAT TAC TTC CAA TGT GAG TGG AAG TGA					

2871	2880	2889	2898	2907	2916
CAT GTG CAA TTT CTA TAC CTG GCT CAT AAA ACC CTC CCA TGT GCA GCC TTT CAT					

2925	2934	2943	2952	2961	2970
GTT GAC ATT AAA TGT GAC TTG GGA AGC TAT GTG TTA CAC AGA GTA AAT CAC CAG					

2979	2988	2997	3006	3015	3024
AAG CCT GGA TTT CTG AAA AAA CTG TGC AGA GCC AAA CCT CTG TCA TTT GCA ACT					

3033	3042	3051	3060	3069	3078
CCC ACT TGT ATT TGT ACG AGG CAG TTG GAT AAG TGA AAA ATA AAG TAC TAT TGT					

3087	3096	3105	3114	3123	3132
GTC AAG AAA					

AAA A 3'

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Figure 3: Protein Sequence for 101P3A11.

MVDPNGNESSATYFILIGLPGLEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDILI
STSSMPKMLAIFWFNSTTIQFDACLLQIFAHSLSGMESTVLLAMAFDRYVAICHPLRHATVTLPRVTKIGV
AAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDDIRVNVVYGLIVIISAIGLDSLLISFSYL
LILKTVLGLTREAQAKAFGTCVSHVCASFIFYVPFIGLSMVHRFSKRRDSPLPVILANIYLLVPPVLPNPIVYG
VKTKEIRQRILRLFHVATHASEP

101P3A41

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Figure 4

Alignment of 101P3A11 (Sbjct) with mouse olfactory receptor S25 (Query)

Query: 34 GNYTVVTEFILLGLTDDITSVILFVMFLIVYSVTLMGNLNIIVLIRTSPQLHTPMYLFL 93
GN + T FIL+GL L +Y + ++GNL II ++RT LH PMY+FL

Sbjct: 6 GNESSATYFILIGLPGLEEAQFWLAFFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFL 65

Query: 94 SHLAFLDIGYSSSVTPIMLRGFLRKGTFIPVAGCVAQLCIVVAFGTSESFLLASMAYDRY 153
L+ +DI S+S P ML F T I C+ Q+ + + ES +L +MA+DRY

Sbjct: 66 CMLSGIDILISTSSMPKMLAIFWFNSTTIQFDACLLQIFAHSLSGMESTVLLAMAFDRY 125

Query: 154 VAICSPLLYSTQMSSTVCILLVGTSYLGWWVNAIFTGCSLNLSFCGPNKINHFFCDYSP 213
VAIC PL ++T ++ + + + G L FC N ++H +C +

Sbjct: 126 VAICHPLRHATVLTLPRTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQD 185

Query: 214 LLKLSCSHDFSFEVIPAISGSIIVVTVFIIALSYVYILVSILKMRSTEGRQKAFSTCTS 273
++KL+C V I S I + +I+ SY+ IL ++L + + E + KAF TC S

Sbjct: 186 VMKLACDDIRVNYYGLIVIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVS 244

Query: 274 HLTAVTLFFGTITFIYVMPQSSYSTDQNK---VVSVFYTVVIPMLNPLIYSFRNKEVKE 329
H+ AV +F+ + FI + +S ++ +++ Y +V P+LNP++Y + KE+++

Sbjct: 245 HVCAVFIFY--VPFIGLSMVHRFSKRRDPLPVILANIYLLVPPVLPNPIVYGVKTKEIRQ 302

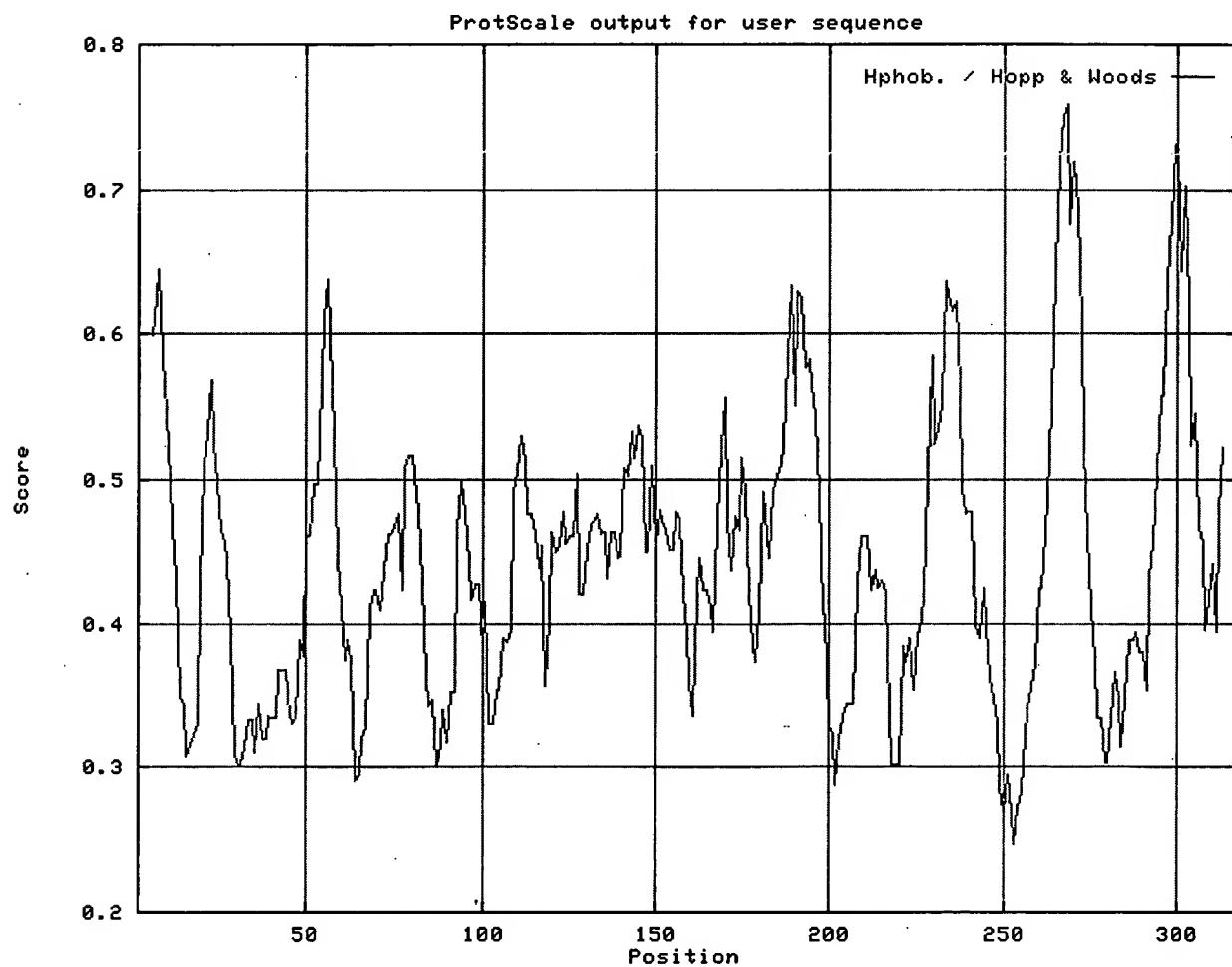
Query: 330 AMKKL 334

+ +L

Sbjct: 303 RILRL 307

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Figure 5:
101P3A11 Hydrophilicity profile
(Hopp T.P., Woods K.R., 1981. Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828)



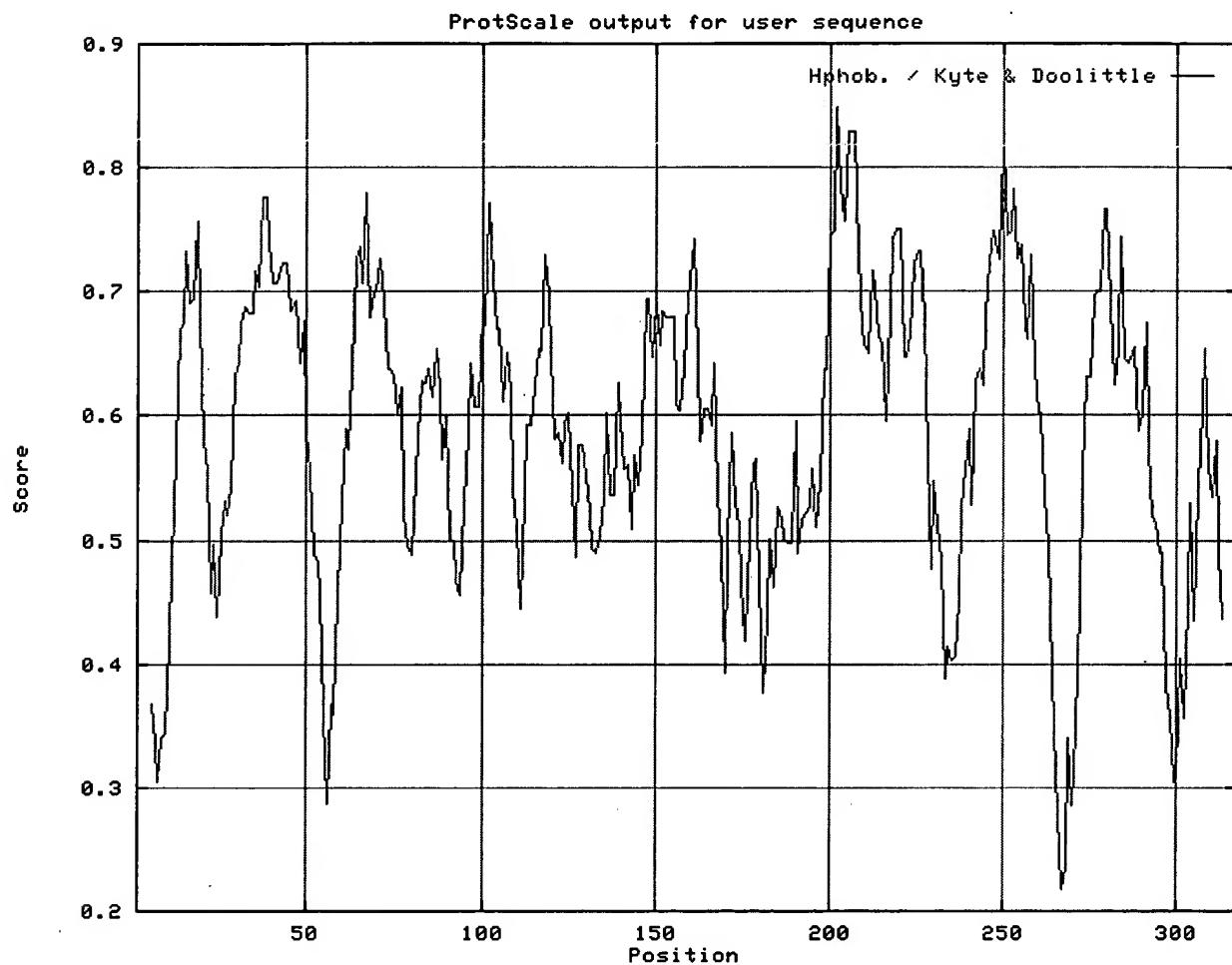
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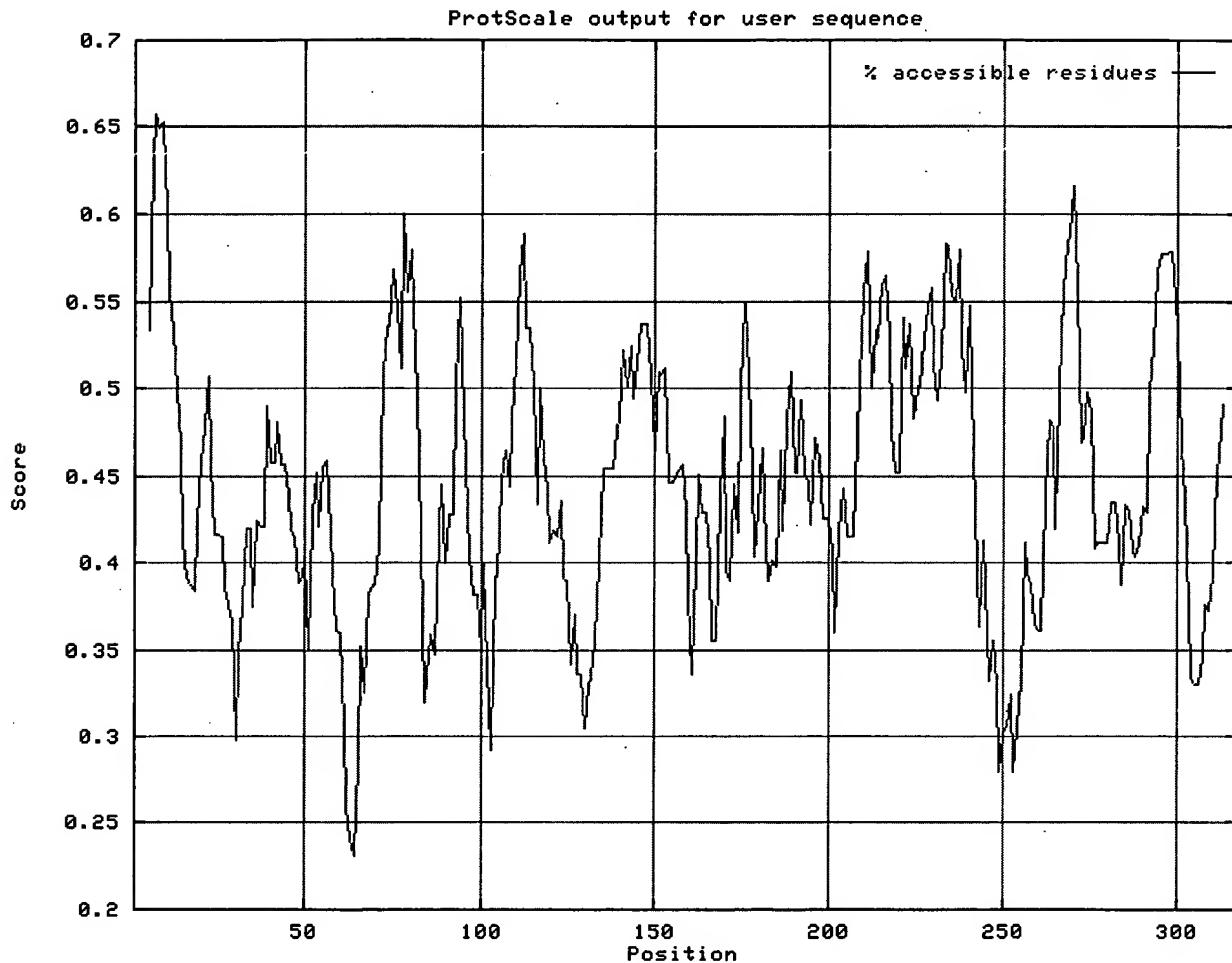
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Figure 6:
101P3A11 Hydropathicity Profile
(Kyte J., Doolittle R.F., 1982. J. Mol. Biol. 157:105-132)



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Figure 7:
101P3A11 % Accessible Residues Profile
(Janin J., 1979. Nature 277:491-492)



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Figure 8:
101P3A11 Average Flexibility Profile
(Bhaskaran R., Ponnuswamy P.K., 1988.
Int. J. Pept. Protein Res. 32:242-255)

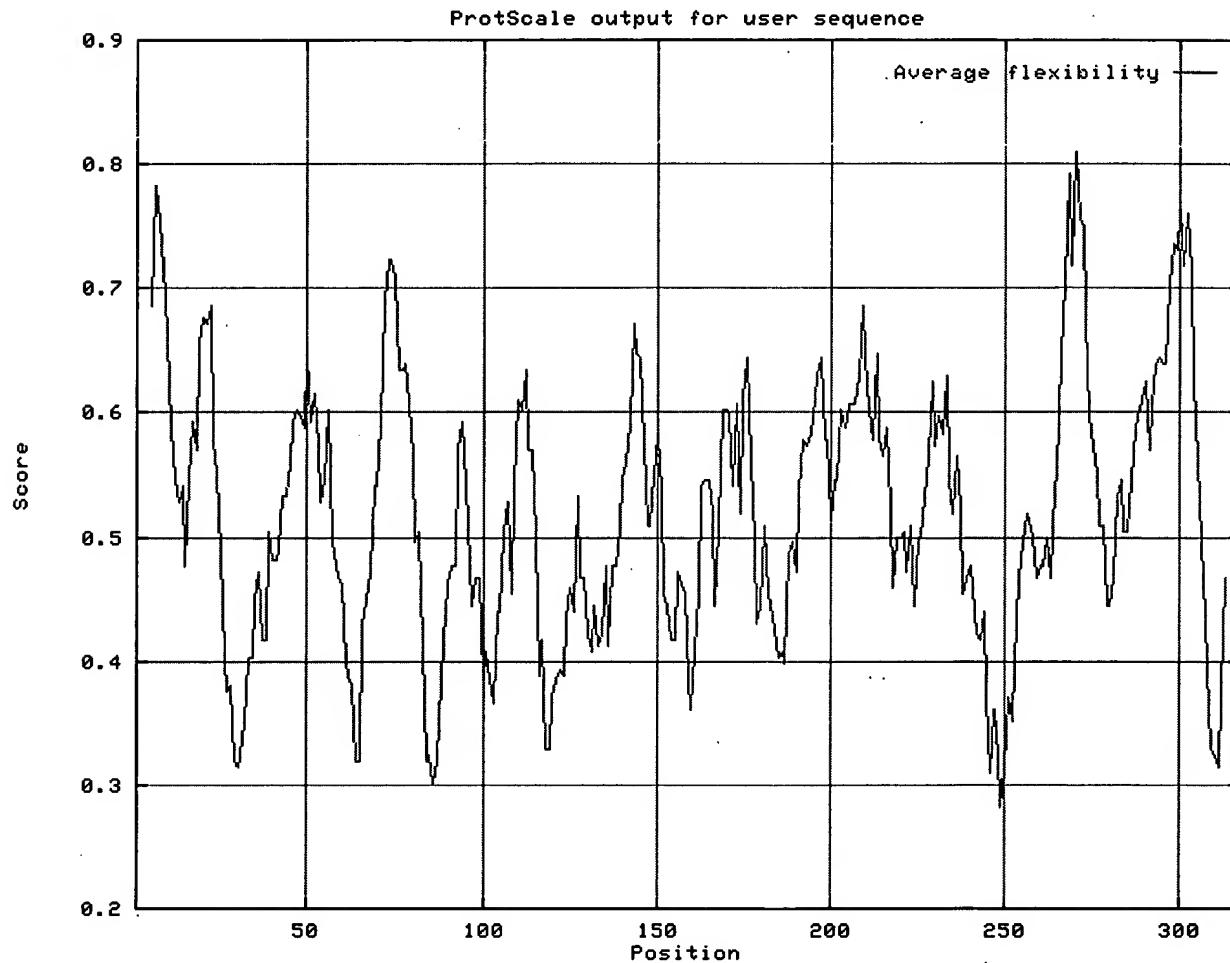
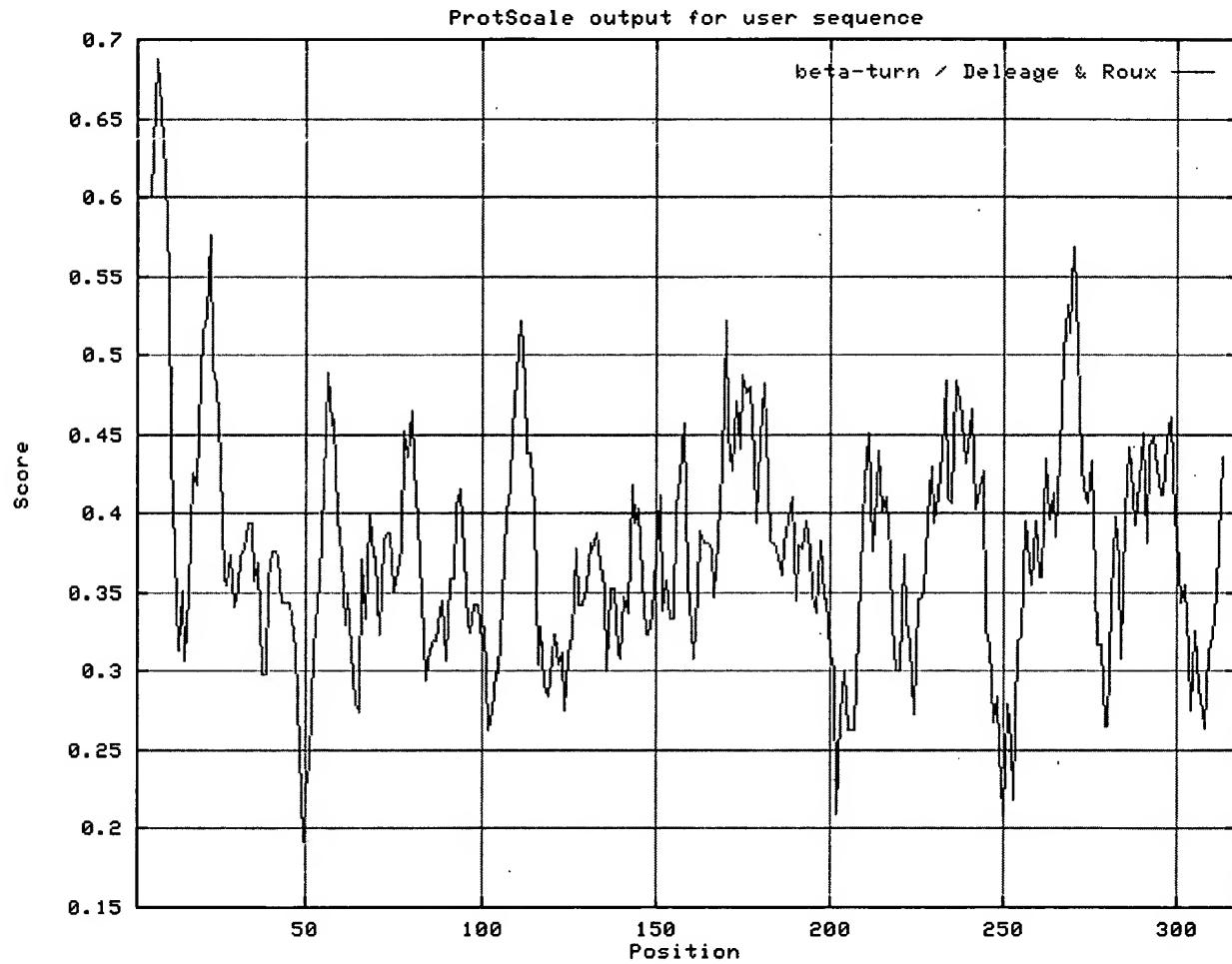


Figure 9:
101P3A11 Beta-turn Profile
(Deleage, G., Roux B. 1987. Protein Engineering 1:289-294)



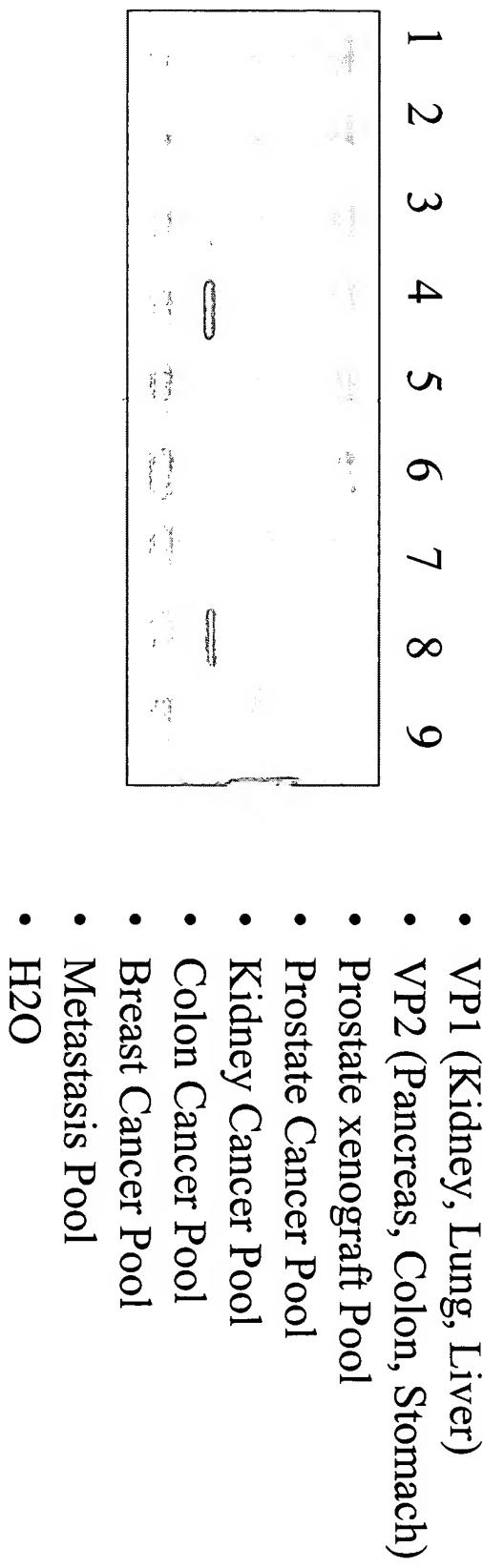
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Figure 10A. Expression of 101P3A11 by RT-PCR



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Figure 10B

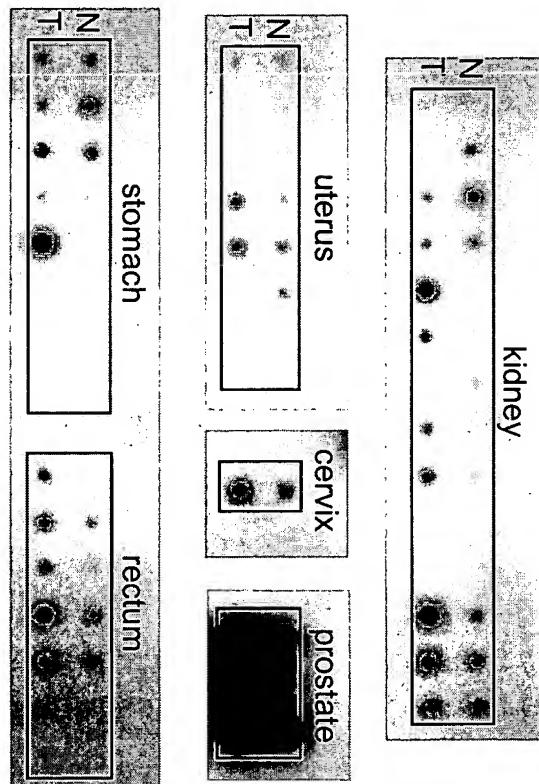
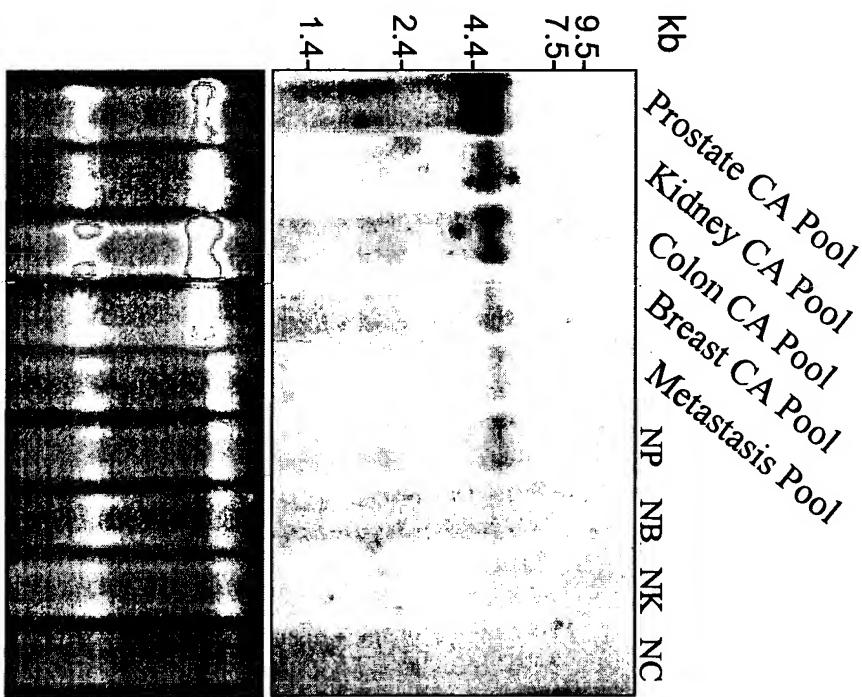


Figure 11. Expression of 101P3A41 in Human Patient Cancer Specimens



10 μ g total RNA per lane from a pool of 3 tumors as follows:

Prostate Cancer Pool = gleason 6, 8, 9

Kidney Cancer Pool = grade 2, 2, 3

Colon Cancer Pool = stage II, III, IV

Breast Cancer Pool = grade 1, 2, 3

Metastasis Pool = colon to lung, colon to liver, ovary to fal. tube

NP = Normal Prostate

NB = Normal Bladder

NK = Normal Kidney

NC = Normal Colon

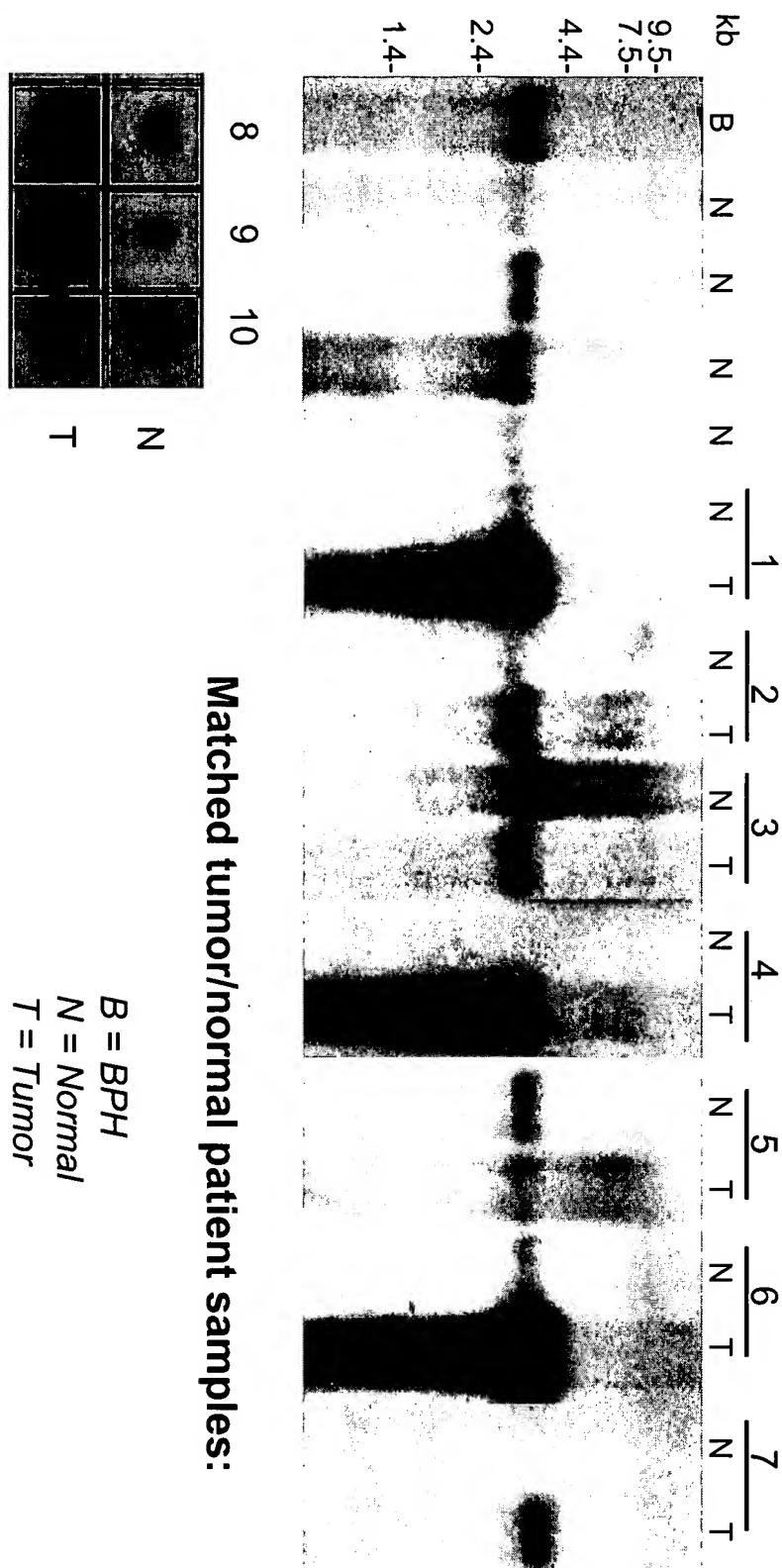
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Figure 12A



Matched tumor/normal patient samples:

Figure 12B and 12C

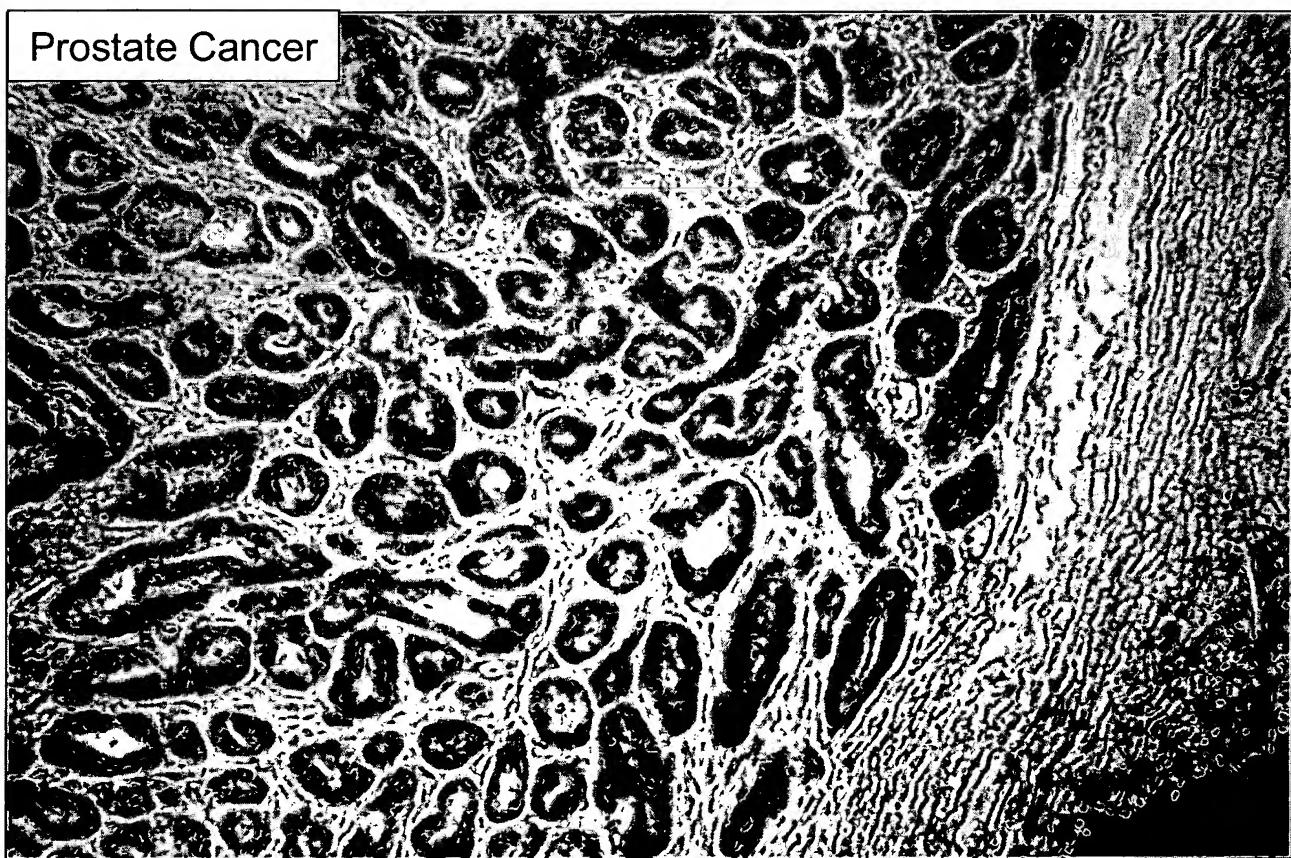
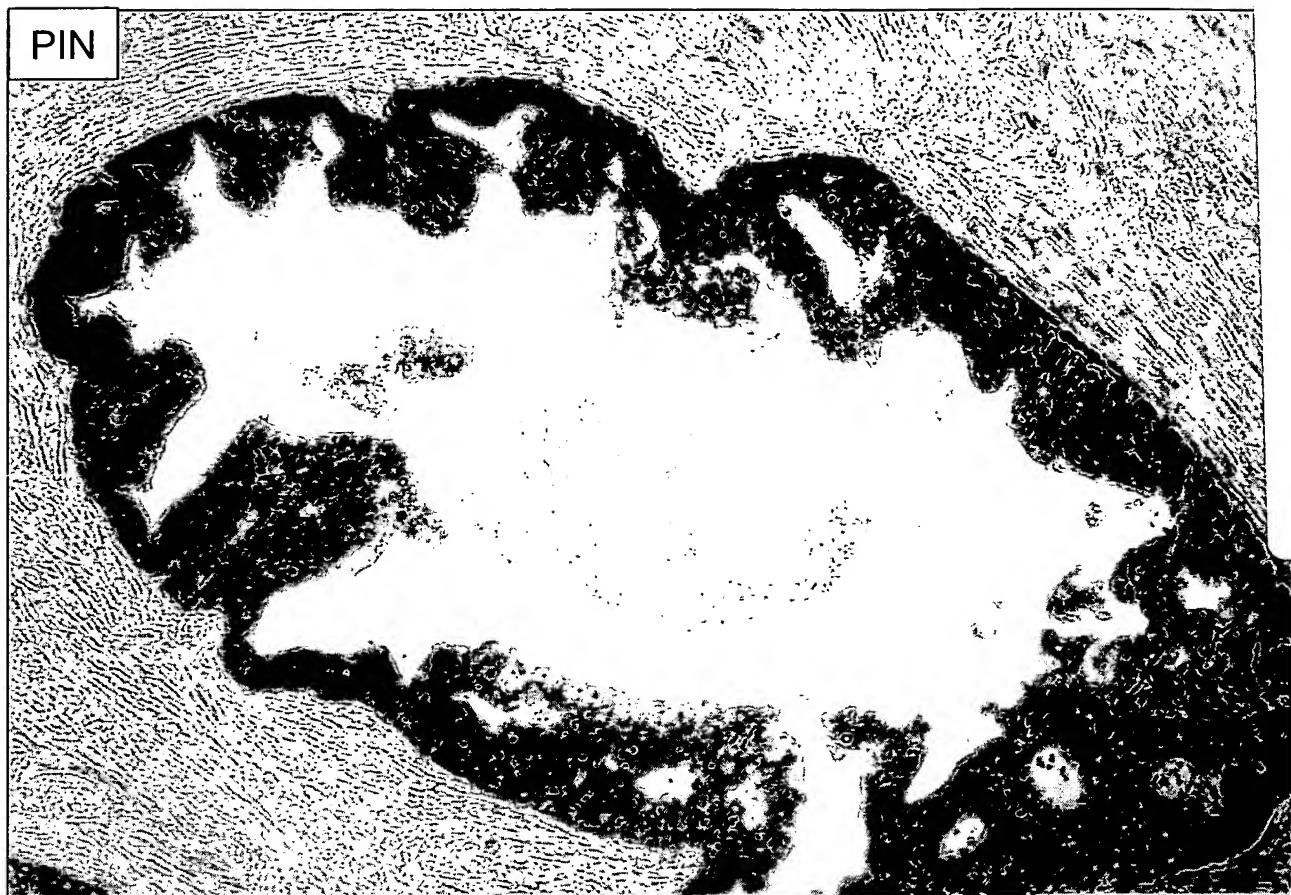
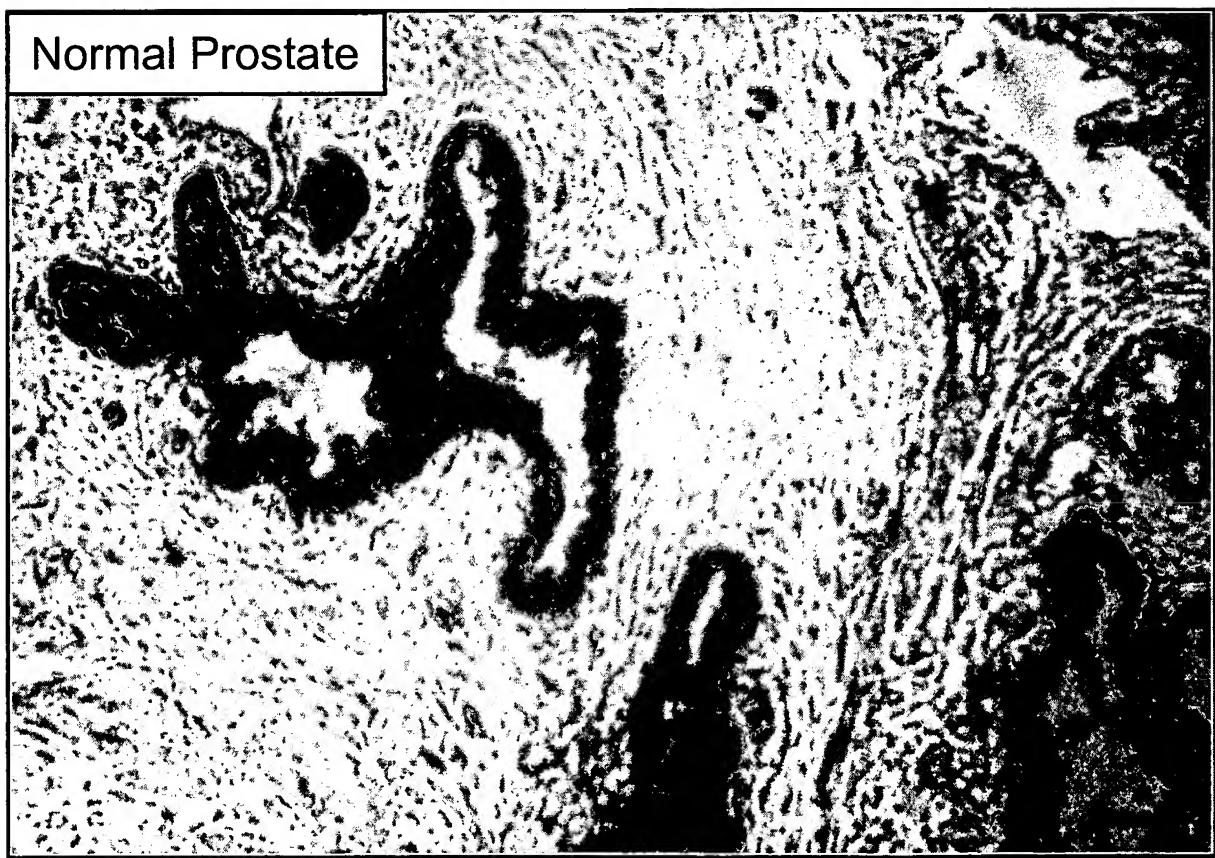
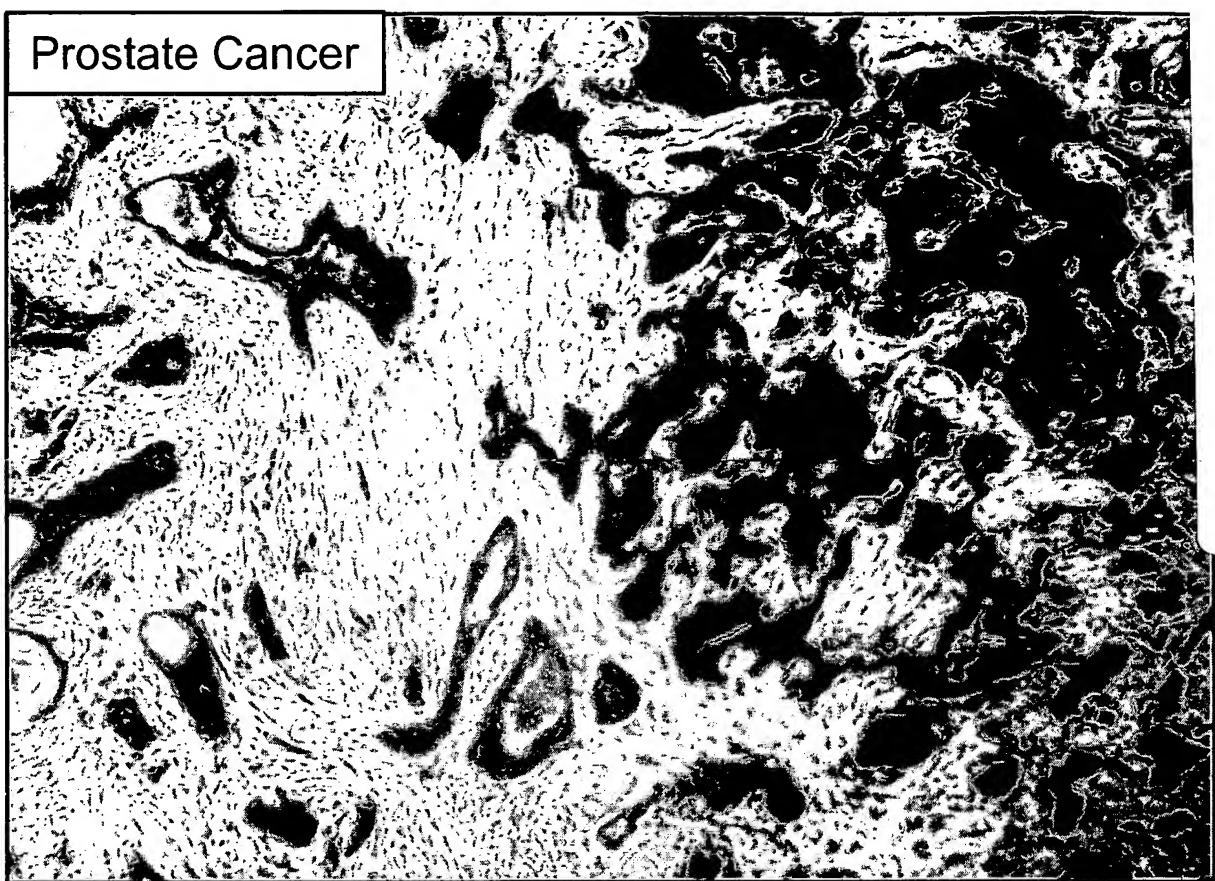


Figure 12D and 12E



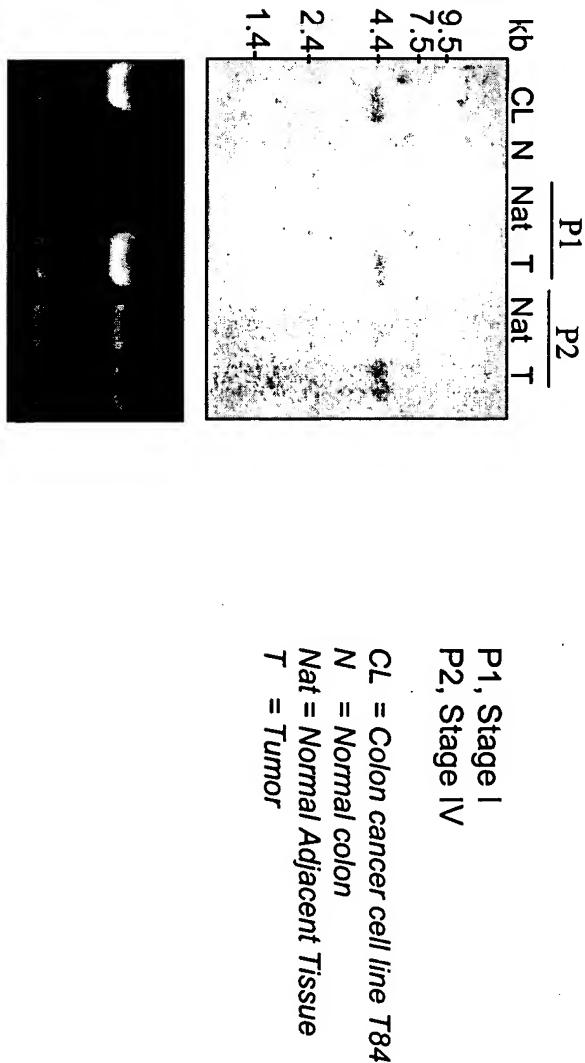
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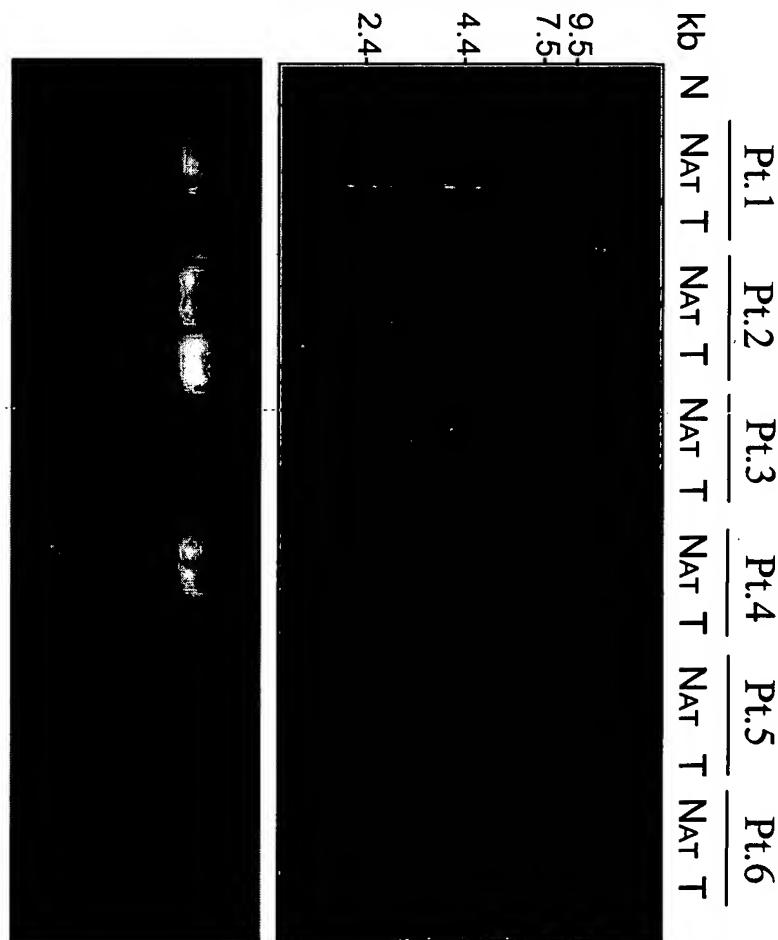
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Figure 13. Expression of 101P3A11 in Colon Cancer Patient Specimens





Pt.1, Papillary carcinoma, stage I
Pt.2, Invasive papillary carcinoma
Pt.3, Clear cell type grade 1/3, focally 2/3
Pt.4, Clear cell type, stage III
Pt.5, Clear cell type, stage III
Pt.6, Clear cell type, stage III

N = Normal kidney
NAT = Normal adjacent tumor
T = Tumor

Figure 14. Expression of 101P3A41 in Kidney Cancer Patient Specimens

Figure 15A-15C. Androgen Regulation of 101P3A11 in Tissue Culture Cells

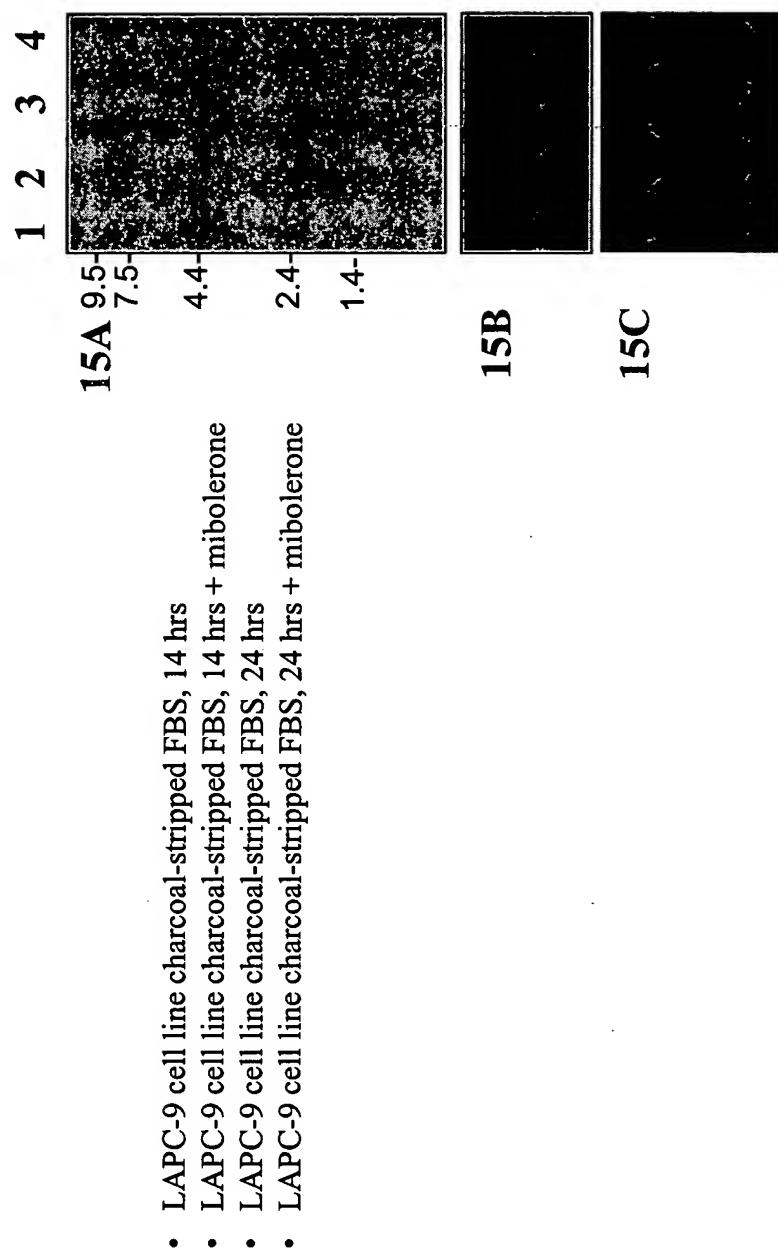
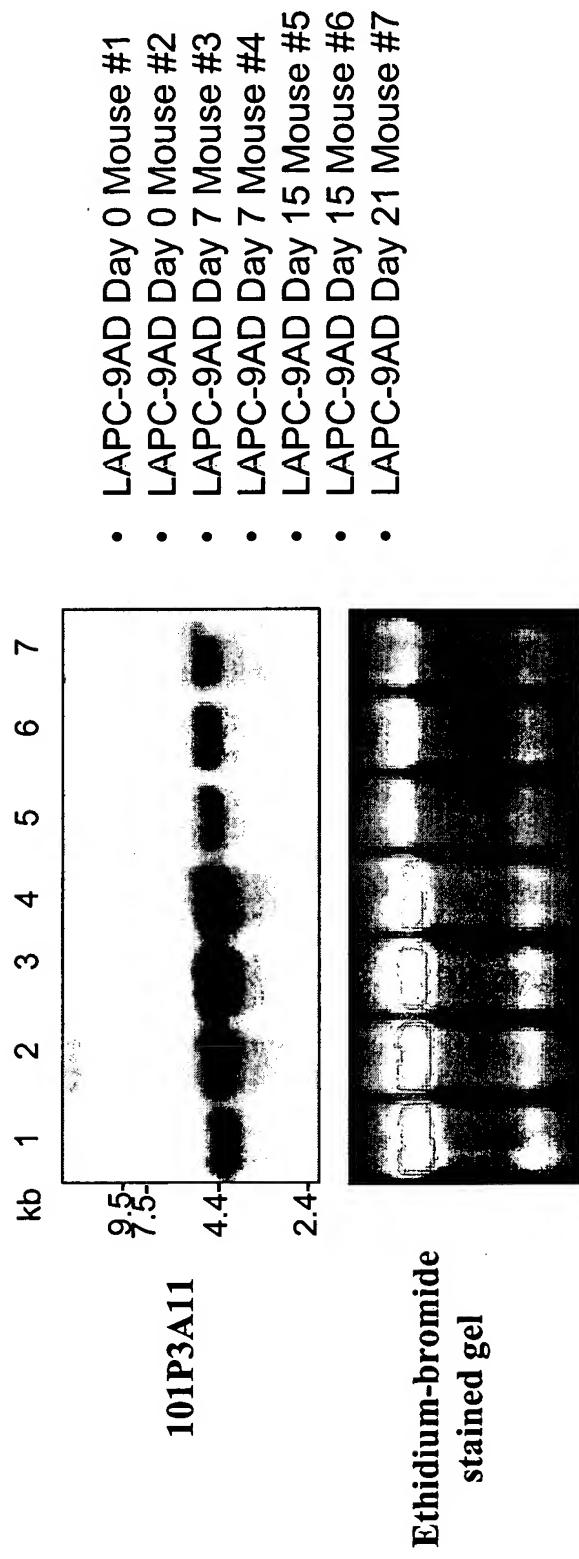


Figure 16. Androgen Regulation of 101P3A11 *In Vivo*



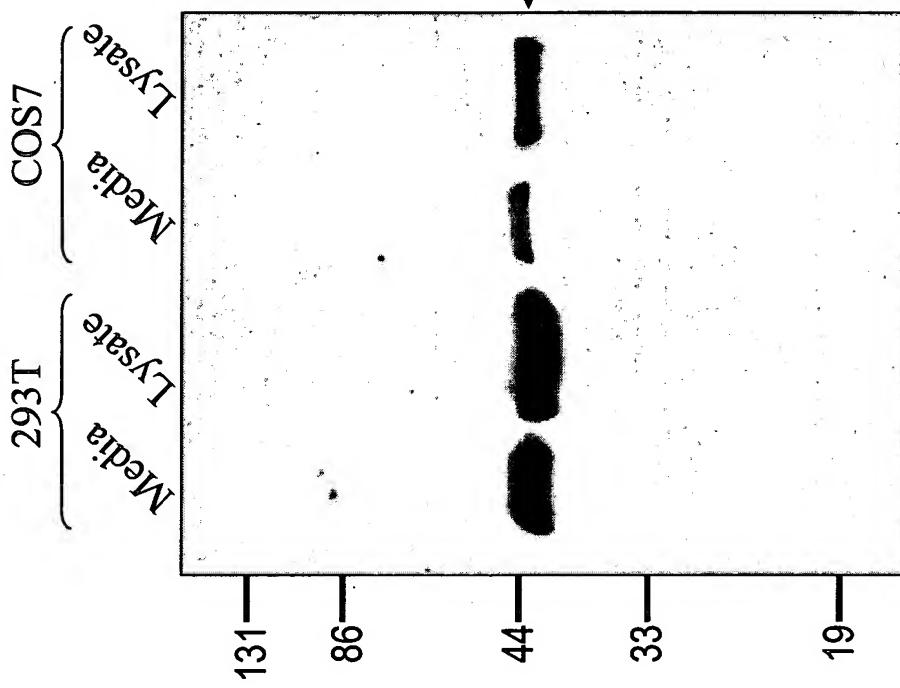
Title: NUCLEIC ACID AND CORRESPONDING PROTEIN
ENTITLED 101P3A41 USEFUL IN TREATMENT AND
DETECTION OF CANCER

First Inventor: Daniel E. H. AFAR, et al

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Figure 17. Expression and Detection of 101P3A11(159-202)-psecFc Fusion Protein



101P3A11(159-202)-psecFc
Fusion Protein

Figure 18. Expression of 101P3A11 in 300.19 Cells

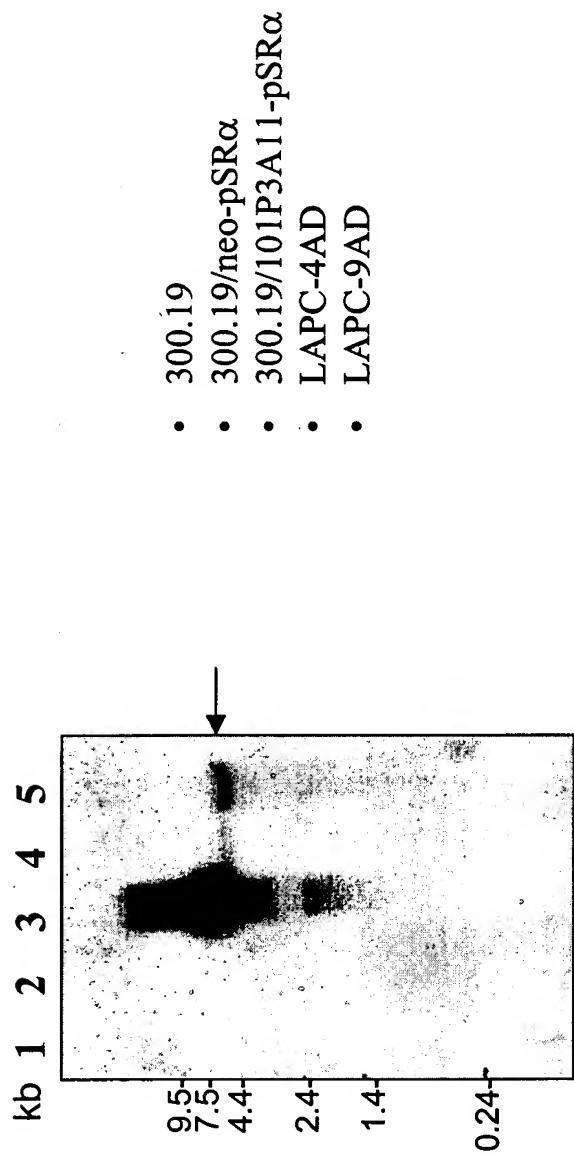


Figure 19A. Secondary structure prediction of 101P3A11

10 20 30 40 50 60 70
| | | | | |
MVDPPNGNESSATYFILLIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLLHEPMYIFLCMLSG
cccccccccceeeeeccchhhhhhhccchhhhhcc
IDILISTSSMPKMLIAIFWFENSTTIQFDACLLQIAHSLSGMESTVLLAMAEDRYVAICHPLRHATVLT
ccccccccchhhheeeccccccceehhhhhhhccchhhhhcc
PRVTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDDIRVNVVYGLIVIISAG
ccchehhhhhhhhccchhhhecc
LDSSLISFSYLLIKTVLGLTREAQAKAKAFGTCVSHVCAVFIFYVPPFIGLSMVKRRDSPLPVILANI
hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhcc
YLLVPPVLNPIVYGVKTKERQRILRLFHATHASEP
hhhhcc

c: random coil (30.60%)
e: extended strand (21.45%)
h: alpha helix (47.95%)

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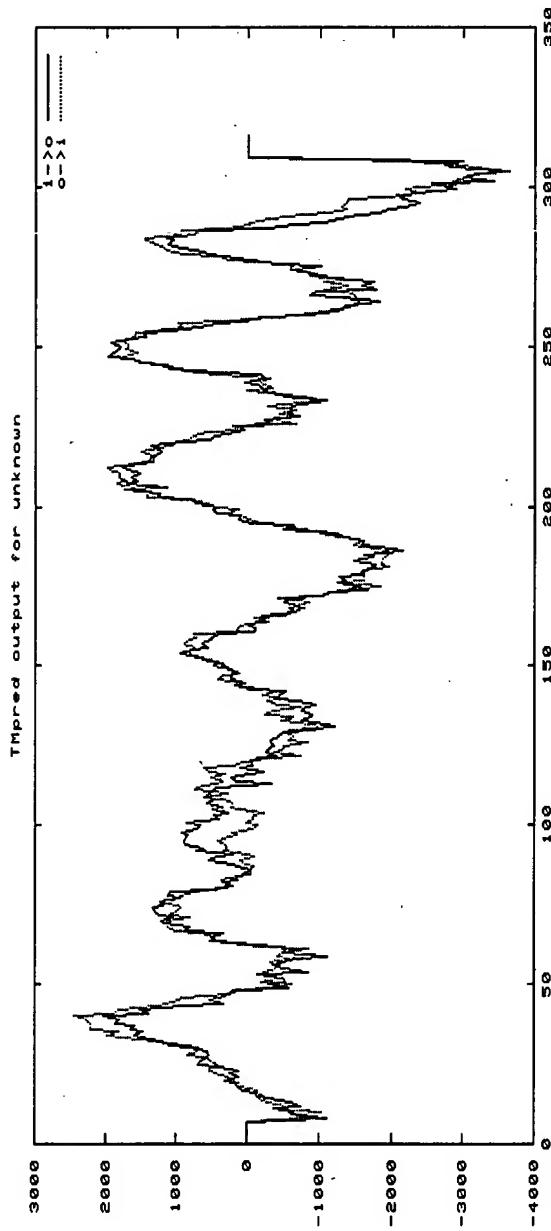
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Figure 19B-19C. Transmembrane prediction of 101P3A11

19B



19C

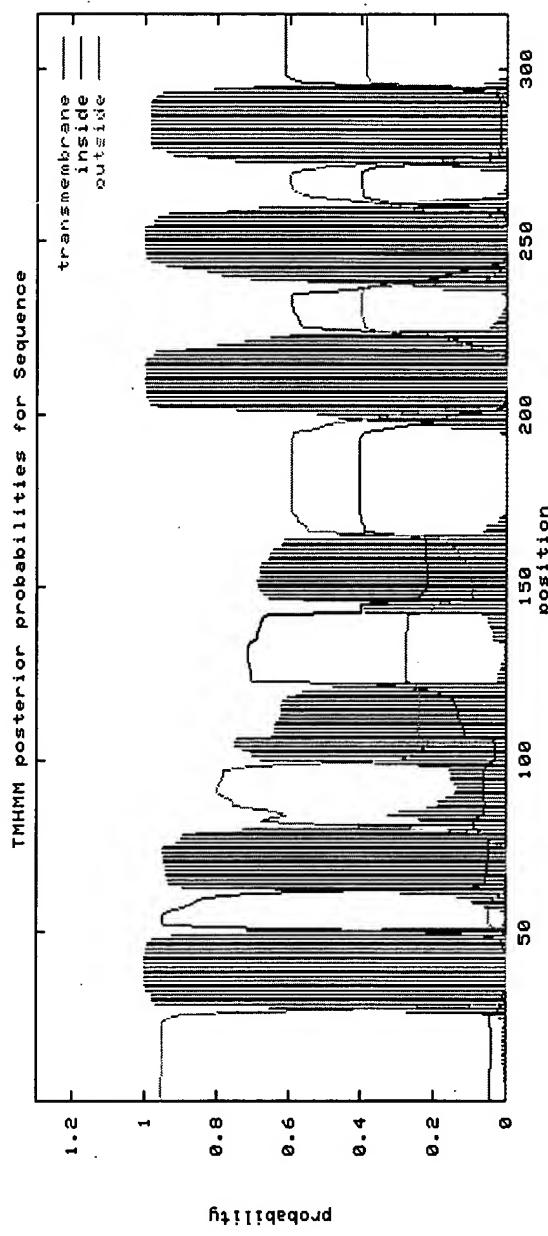


Figure 20. Expression of 101P3A11 in NIH-3T3 Tumors

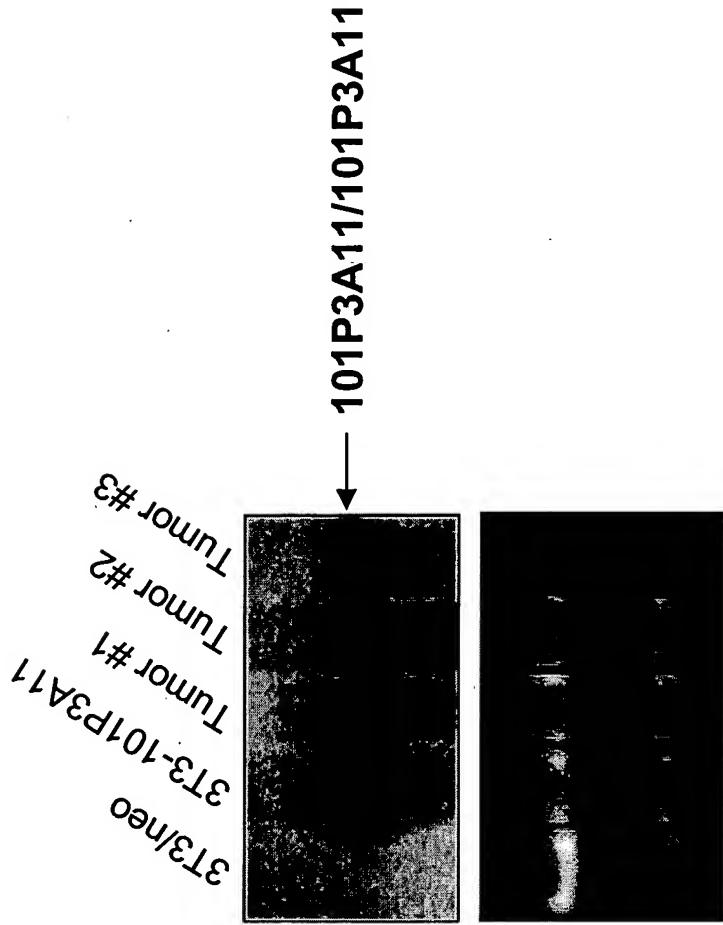
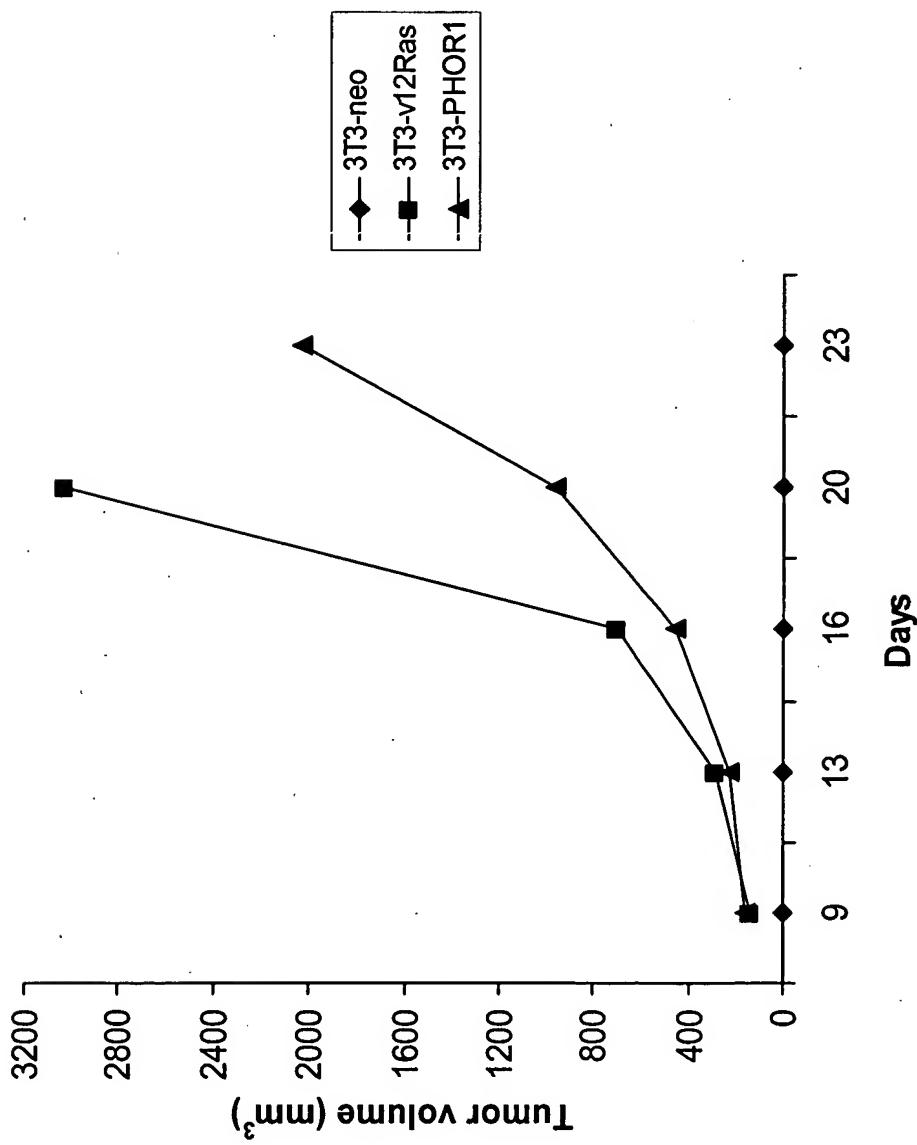
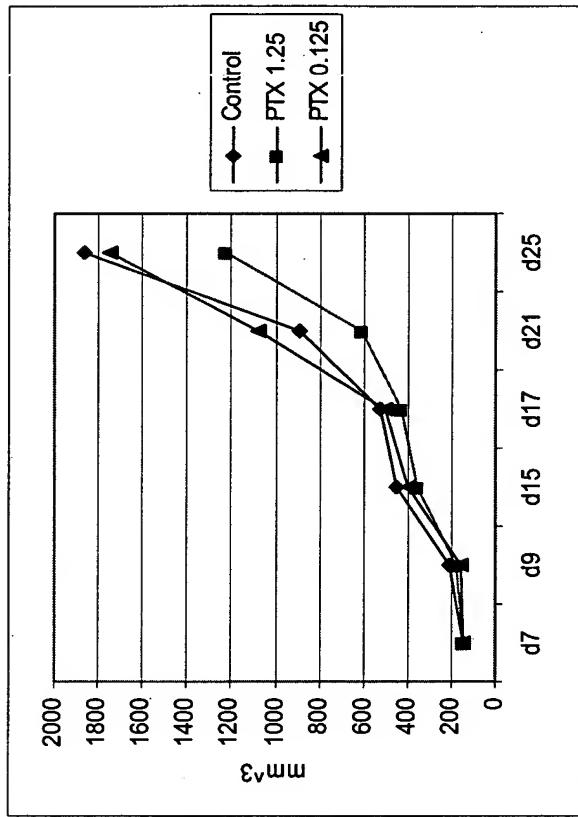


Figure 21: 101P3A11 Induces Tumor Formation of 3T3 Cells



- Injection of 10^6 3T3-neo, 3T3-Ras or 3T3-101P3A11 cells subcutaneously into SCID mice revealed that 6/6 3T3-Ras-injected mice formed tumors, 6/6 3T3-101P3A11-injected mice formed tumors, and 0/6 3T3-neo-injected mice formed tumors.

Figure 22: PTX Reduces the *in vivo* Growth of 3T3-101P3A11 Tumors



- Pertussis toxin inhibits the sub-cutaneous growth of 3T3-101P3A11 tumors in SCID mice.
- The inhibitory activity of pertussis toxin occurs in a dose dependent manner.

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Figure 23: Alignment of 101P3A11-PHOR-1 with the rat GPCR RA1C (gi|3420759).

Identities = 179/299 (59%), Positives = 231/299 (76%), Gaps = 1/299 (0%)

PHOR: 14 FILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDI 73
F+LIG+PGLEEA FW FPL S+Y +A+ GN +++IVRTE SLH PMY+FLCML+ ID+
RA1C: 11 FMLIGIPGLEEAHFWFGFPLLSMYAVALFGNCIVVFIVRTERSLHAPMYLFLCMLAAIDL 70

PHOR: 74 LISTSSMPKMLAIFWFNSTTIQFDACLLQIFAHSLSGMESTVLLAMAFDRYVAICHPLR 133
+STS+MPK+LA+FWF+S I FDACL Q+F IH+LS +EST+LLAMAFDRYVAICHPLR
RA1C: 71 ALSTSTMPKILALFWFDSREITFDACLAQMFFIHSAIESTILLAMAFDRYVAICHPLR 130

PHOR: 134 HATVLTLPRVTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLA 193
HA VL +IG+ A+VRG+ PLP+ IK+L FC SN+LSHSYC+HQDVMKLA D
RA1C: 131 HAAVLNNNTVTQIGMVALVRGSLFFFPLPLLKRLAFCHSNVLSHSYCVHQDVMKLA 190

PHOR: 194 IRVNVVYGLIVIISAIGLDSLISFSYLLILKTVLGL-TREAQAKAFGTCVSHVCASFIF 252
NVVYGL I+ +G+D + IS SY LI++ VL L ++ +AKAFGTCVSH+ V F
RA1C: 191 TLPNVVYGLTAILLVMGVDFVMFISLSYFLII RAVLQLPSKSERAKAFGTCVSHIGVVLAF 250

PHOR: 253 YVPFIGLSMVHRFSKRRDPLPVILANIYLLVPPVLPNPIVYGVKTKEIRQRILRLFHVA 311
YVP IGLS+VHRF D + V++ ++YLL+PPV+NPI+YG KTK+IR R+L +F ++
RA1C: 251 YVPLIGLSVVHRFGNSLDPIVHVLMGDVYLLLPPVINPIIYGAKTKQIRTRVLAMFKIS 309

Figure 24: Alignment of 101P3A11-PHOR-1 with the human prostate specific GPCR. (gi|13540539)

Identities = 179/299 (59%), Positives = 233/299 (77%), Gaps = 1/299 (0%)

PHOR: 14 FILIGLPGLEEAQFWLAFLPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDI 73
F+LIG+PGLE+A FW+ FPL S+Y++A+ GN +--+IVRTE SLH PMY+FLCML+ ID+

GPCR: 11 FVLIGIPGLEKAHFVWGVFPPLSMYVVAMFGNCIVVFIVRTERSLHAPMYLFLCMLAAIDL 70

PHOR: 74 LISTSSMPKMLAIFWFNSTTIQFDACLLQIFAHSLSGMESTVLLAMAFDRYVAICHPLR 133
+STS+MPK+LA+FWF+S I F+ACL Q+F IH+LS +EST+LLAMAFDRYVAICHPLR

GPCR: 71 ALSTSTMPKILALFWFDSREISFEACLTQMFFIHALSESTILLAMAFDRYVAICHPLR 130

PHOR: 134 HATVLTLPRVTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLA CDD 193
HA VL +IG+ AVVRG+ PLP+ IK+L FC SN+LSHSYC+HQDVMKLA D

GPCR: 131 HAAVLNNNTVTAQIGIVAVVRGSLFFFPLPLLKRLAFCHSNVLSHSYCVHQDVMKLAYAD 190

PHOR: 194 IRVNVVYGLIVIISAIGLDSLISFSYLLILKTVLGL-TREAQAKAFGTCVSHVCASFIF 252
NVVYGL I+ +G+D + IS SY LI++TVL L ++ +AKAFGTCVSH+ V F

GPCR: 191 TLVPNYYGLTAILLVMGVDMFISLSYFLIIRTVLQLPSKSERAFAFGTCVSHIGVVLAF 250

PHOR: 253 YVPFIGLSMVHRFSKR RDSPLPVILANIYLLVPPVLPNPIIVYGVKTKEIRQRILRLFHVA 311
YVP IGLS+VHRF + V++ +IYLL+PPV+NPI+YG KTK+IR R+L +F ++

GPCR: 251 YVPLIGLSVVHRFGNSLHPIVRVVMGDIYLLLPPVINPIIYGAKTKQIRTRVLAMFKIS 309

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Figure 25: Alignment with human olfactory receptor 5H12 (gi|14423836)

Identities = 163/304 (53%), Positives = 214/304 (69%), Gaps = 1/304 (0%)

PHOR: 7 NESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLC 66
N + +F+L G+PGLE + WL+ PLC +Y +A+ GN I+ VR E SLHEPMY FL

HOR5: 5 NVTHPAFFLLTGIPGLESSHSWLSGPLCVMYAVALGGNTVILQAVRVEPSLHEPMYYFLS 64

PHOR: 67 MLSGIDILISTSSMPKMLAIFWFNSTTIQFDACLLQIFAHSLSGMESTVLLAMAFDRYV 126
MLS D+ IS +++P +L F N+ I FDACL+Q+F IH S MES +LLAM+FDRYV

HOR5: 65 MLSFSDVAISMATLPTVLRFCLNARNITFDACLIQMFLIHFFSMMESGILLAMSFDRYV 124

PHOR: 127 AICHPLRHATVLTLPRTVKIGVAAVVRGAALMAPLPVFIQLPFCRSNILSHSYCLHQDV 186
AIC PLR+ATVLT + +G+ A R + PLP IK+LP CRSN+LSHSYCLH D+

HOR5: 125 AICDPLRYATVLTTEVIAAMGLGAAARSFITLFPLPFLIKRLPICRSNVLSHSYCLHPDM 184

PHOR: 187 MKLACDDIRVNVVYGLIVIISAIQLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVSH 245
M+LAC DI +N +YGL V++S . G+D I SY+LIL++V+ +RE + KA TCVSH

HOR5: 185 MRLACADISINSIYGLFVLVSTFGMDLFFIIFLSYVLILPSVMATASREERLKALNTCVSH 244

PHOR: 246 VCAVFIFYVPFIGLSMVHRFSKRRDSPLPVILANIYLLVPPVLPNPIVYGVKTKEIRQRIL 305
+ AV FYVP IG+S VHRF K + V+++N+YL VPPVLPN++Y KTKEIR+ I

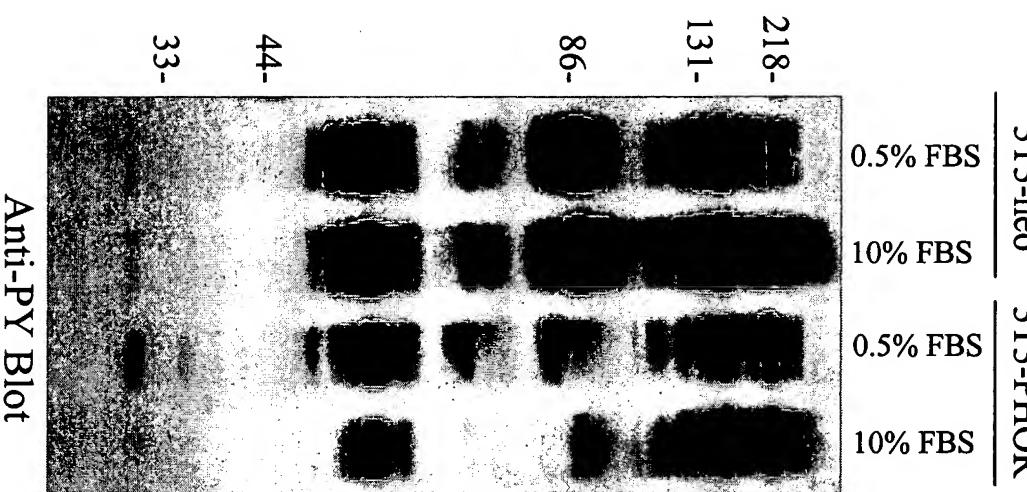
HOR5: 245 ILAVLAFYVPMIGVSTVHRFGKHVPCYIHVLMNSNVYLFVPPVLPNPLIYSAKTKEIRRAIF 304

PHOR: 306 RLFH 309

R+FH

HOR5: 305 RMFH 308

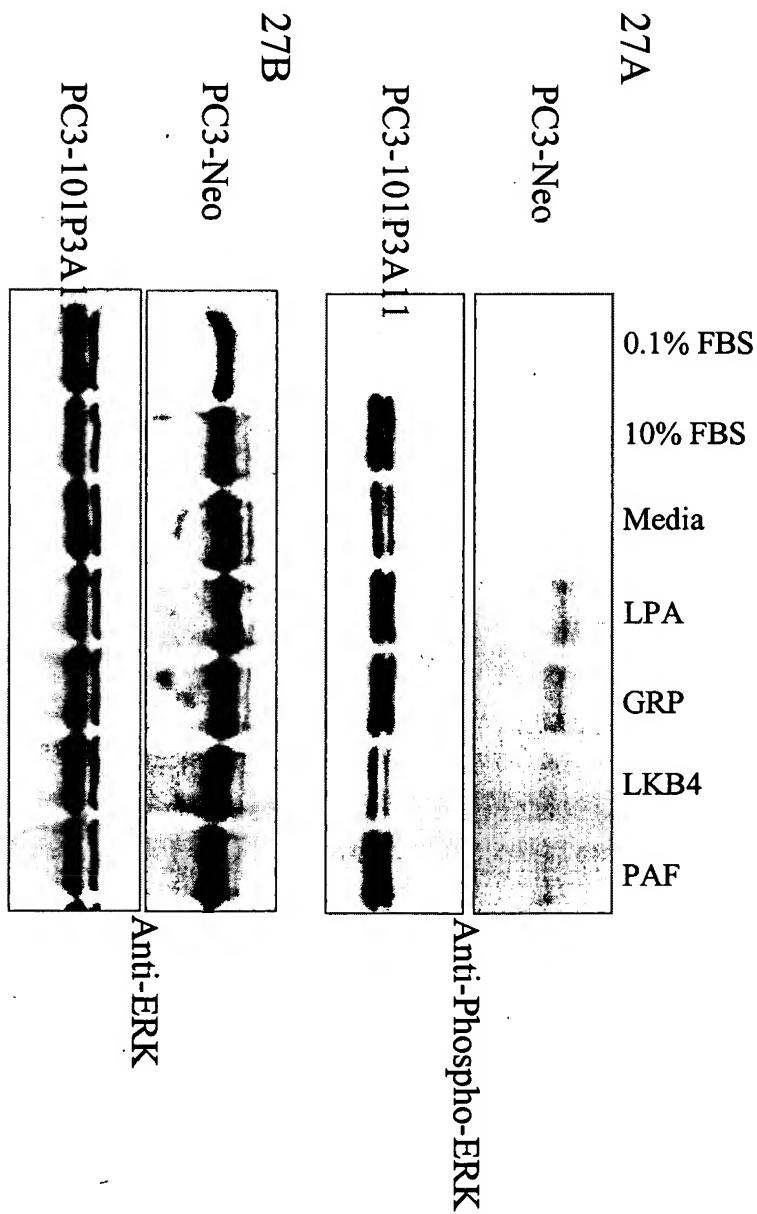
Figure 26: 101P3A11 Modulated Tyrosine Phosphorylation in NIH-3T3 Cells



Anti-PY Blot

- 101P3A11 mediated the de-phosphorylation of proteins at 200, 120-140, 85-90 and 55 kDa
- 101P3A11 induced the phosphorylation of proteins at 80 and 29 kDa in NIH-3T3 cells.

Figures 27A-27B: ERK Phosphorylation by PCR Ligands in 101P3A11
Expressing Cells



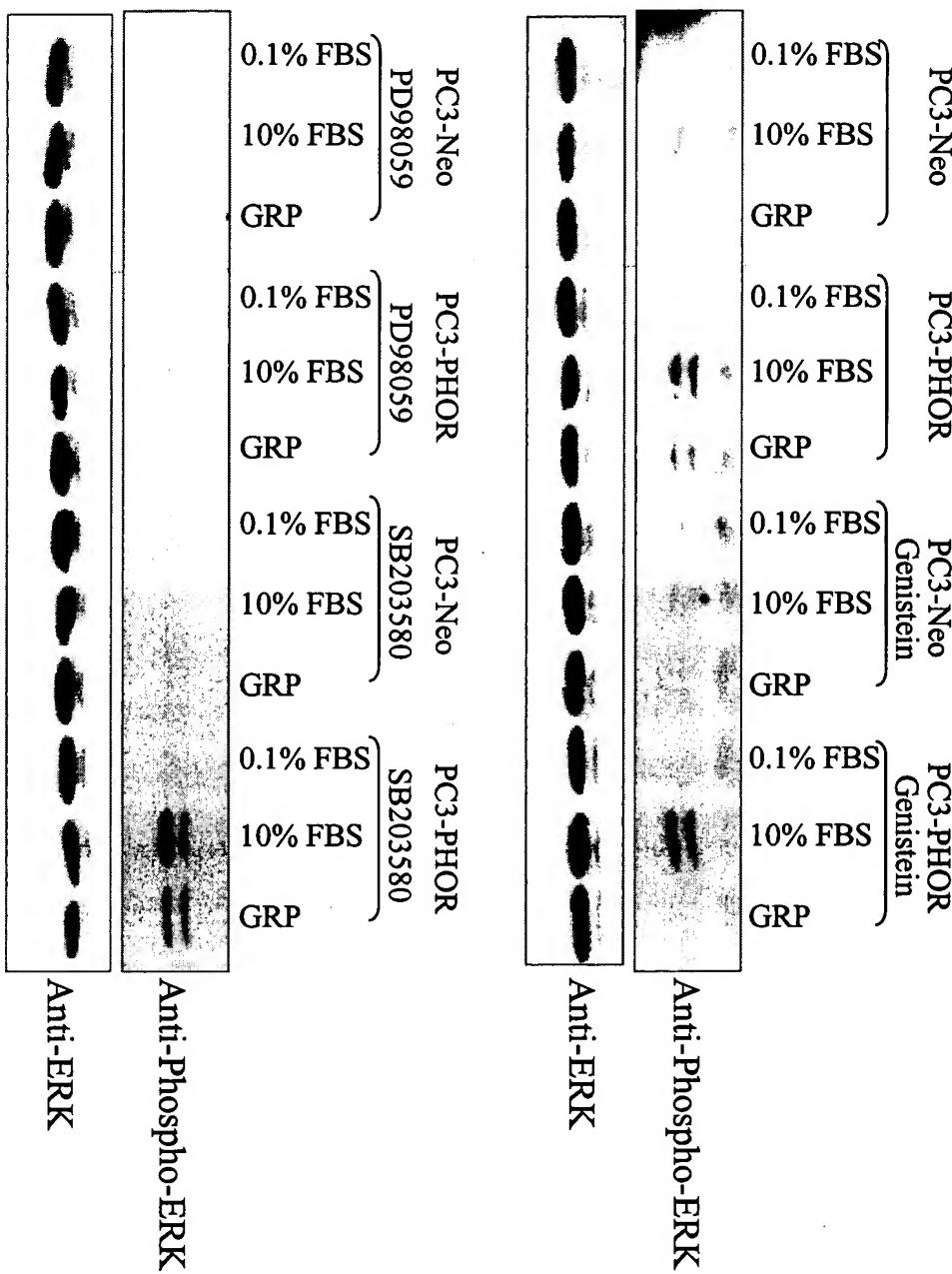
•FBS, lipophosphatidic acid, gastrin releasing peptide, leukotriene and platelet activating factor induced the phosphorylation of ERK in 101P3A11 expressing cells.

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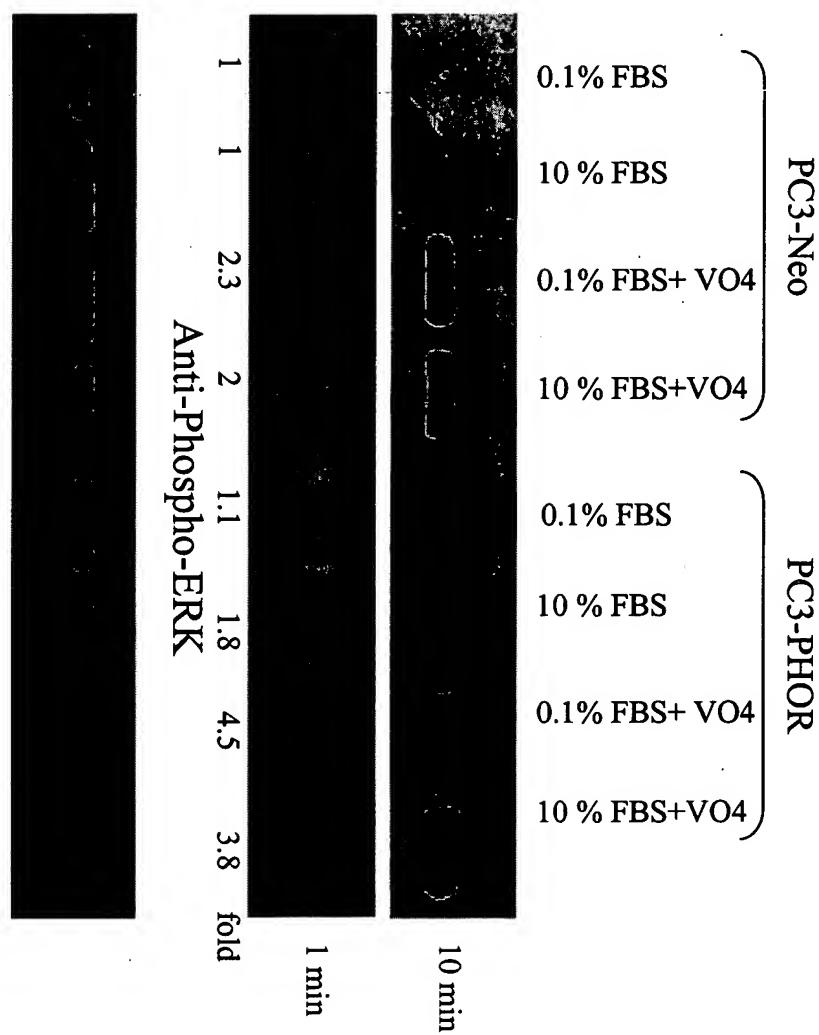
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Figure 28: Inhibition of 101P3A11-Mediated ERK Activation by PD98059



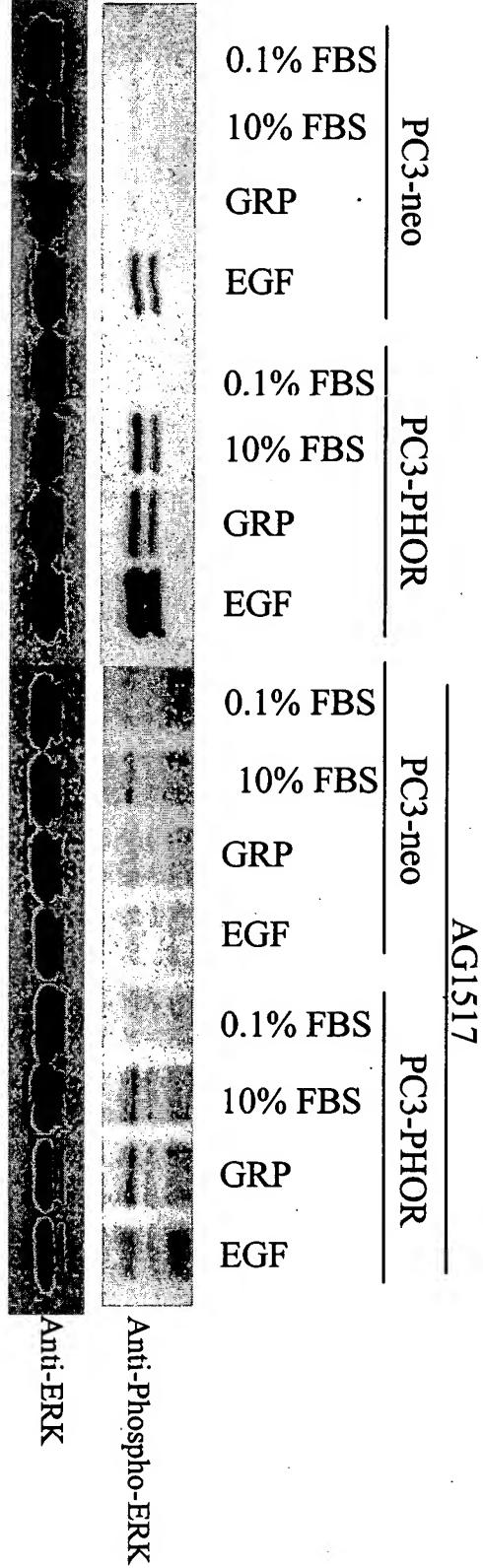
- ERK phosphorylation was inhibited by a MEK specific(PD98059) but not a p38 specific (SB203580) inhibitor in PC3-101P3A11 cells.

Figure 29: Enhanced ERK Phosphorylation in Sodium Orthovanadate Treated
PC3-101P3A11 Cells



•Sodium orthovanadate induced increased ERK phosphorylation in PC3-101P3A11 cells relative to
PC3-neo cells.

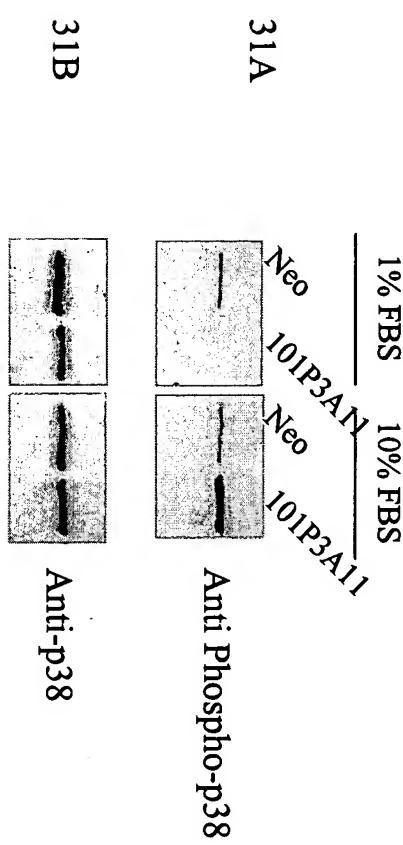
}
**Figure 30: Inhibition of 101P3A11-Mediated ERK Phosphorylation
by AG1517**



- The EGFR inhibitor, AG1517, inhibits EGF-mediated ERK phosphorylation in control and 101P3A11 expressing PC3 cells.
- AG1517 partially inhibits 101P3A11 mediated ERK phosphorylation in PC3 cells.

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Figure 31A-31B: Activation of p38 in PC3-101P3A11 Cells



- Expression of 101P3A11 mediates p38 phosphorylation in cells treated with 10% FBS.

Figure 32: 101P3A11 Induced Accumulation of cAMP in PC3 Cells

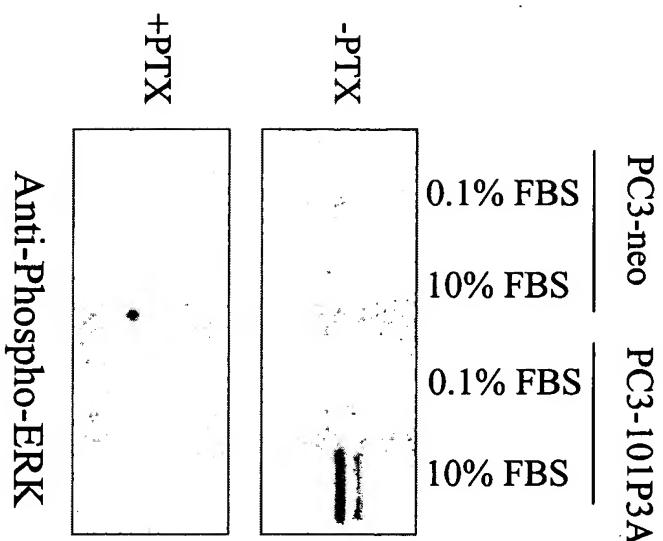
		Fold change in [cAMP]	
		PC3-Neo	PC3-PHOR
0.1%FBS	-PTX	1	4.302
	+PTX	1.403	2.577
10%FBS	-PTX	2.738	6.978
	+PTX	2.163	2.752

Fold Change in cAMP accumulation was calculated relative to PC3-neo cells grown in 0.1%FBS

- Expression of 101P3A11 increased the accumulation of cAMP in cells treated with 0.1% and 10% FBS.
- FBS-induced cAMP accumulation in 101P3A11 cells was inhibited by pertussis toxin.

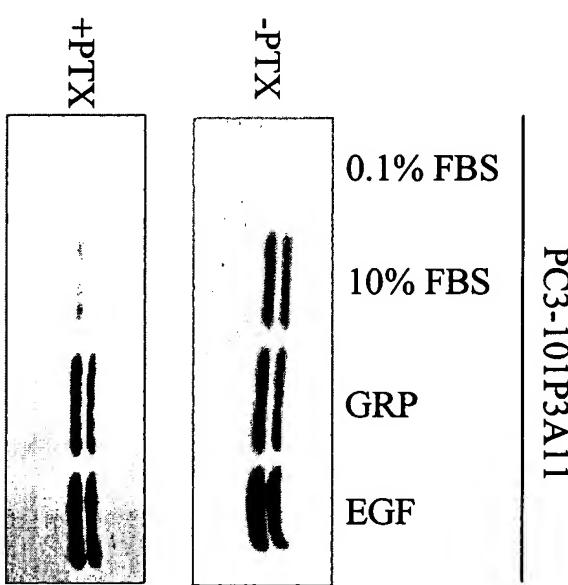
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Figure 33: Pertussis Toxin Inhibits 101P3A11 Mediated ERK
Phosphorylation



- Pertussis toxin inhibited FBS- mediated ERK phosphorylation in 101P3A11 expressing cells.

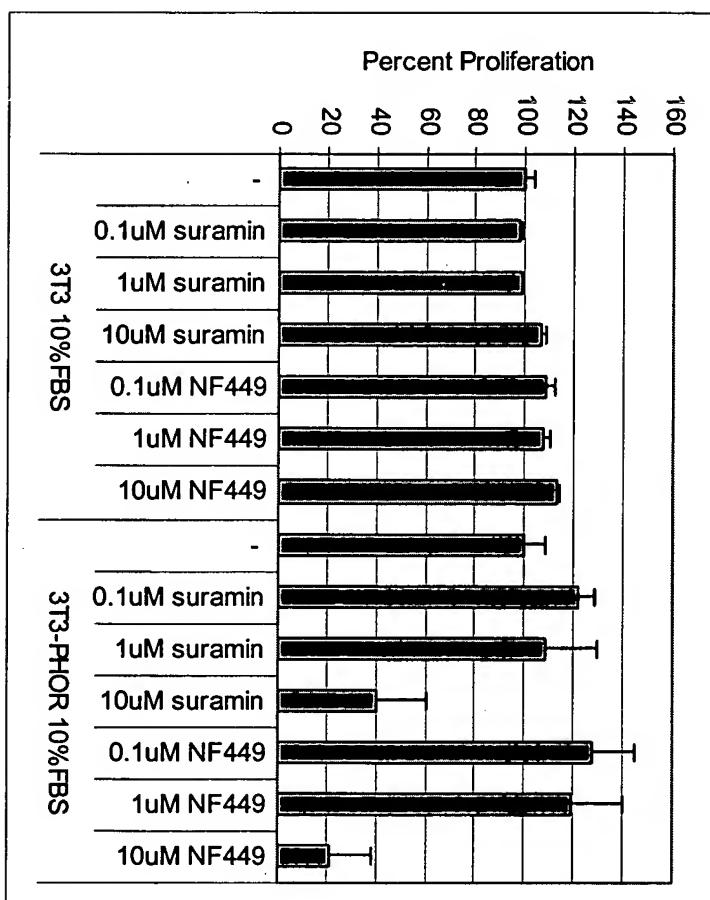
Figure 34: Pertussis Toxin Inhibited ERK Phosphorylation in PC3-
101P3A11 Cells



Anti-Phospho-ERK

- Pertussis toxin inhibited FBS- mediated ERK phosphorylation in 101P3A11 expressing cells.
- The inhibitory activity of pertussis toxin on ERK phosphorylation was more dramatic in FBS-treated than EGF or GRP-treated PC3-101P3A11 cells.

Figure 35: Inhibition of 101P3A11 Mediated Signaling by Suranim



- Control NIH 3T3 and 3T3-101P3A11 cells were grown in the presence of absence of G protein inhibitors suranim and NF449. Proliferation was analyzed by Alamar blue after 72 hours.
- Suranim and NF449 inhibited the proliferation of 101P3A11 expressing but not control cells.

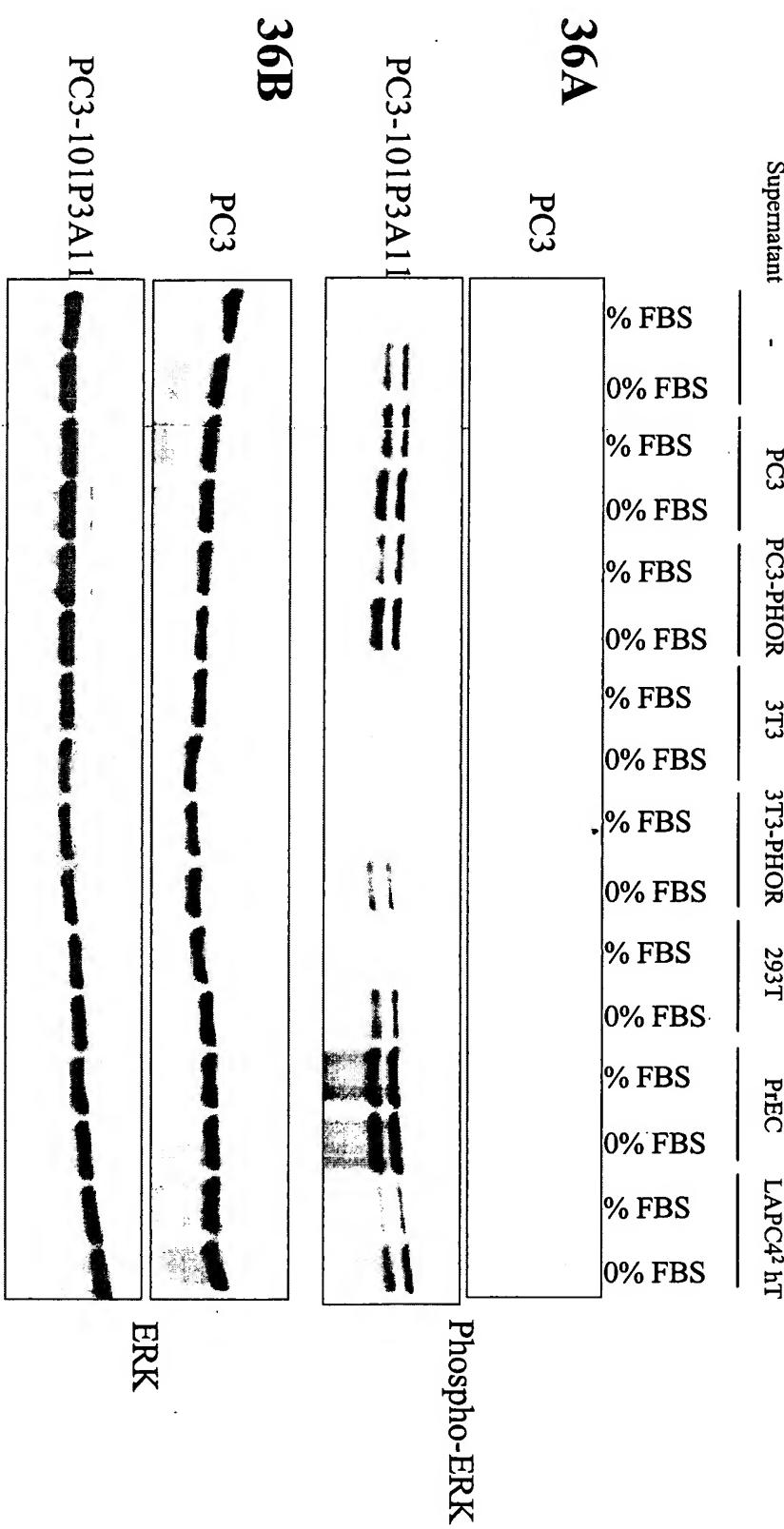
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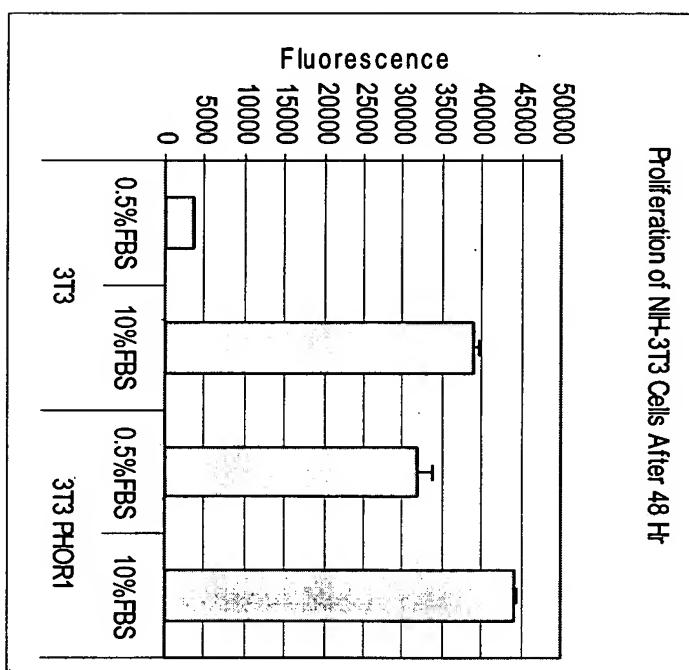
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Figures 36A-36B: 101P3A11 Mediated ERK Phosphorylation By Conditioned Media



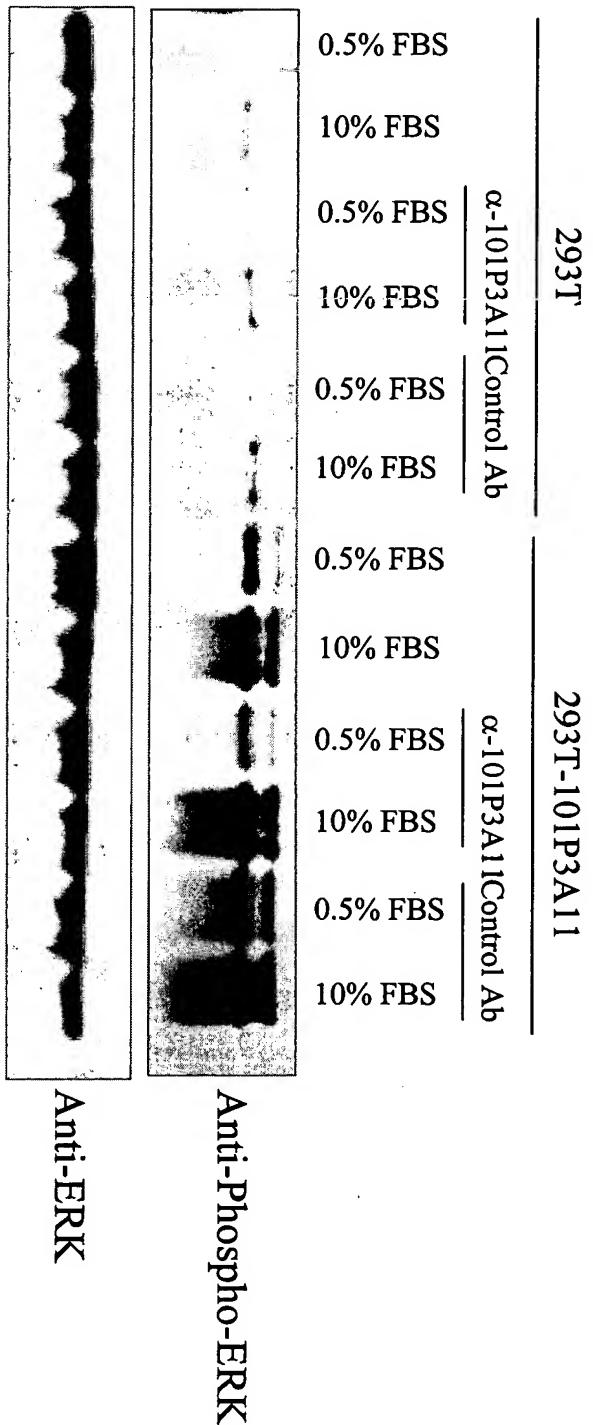
- Supernatants from PC3, PC3-101P3A11, PrEC and LAPC4² cells induce ERK phosphorylation in PC3 101P3A11 but not PC3 cells.
- Supernatants from 3T3 and 293T cells had little specific effect on ERK phosphorylation.

Figure 37: 101P3A11 Enhances The Proliferation of 3T3 Cells



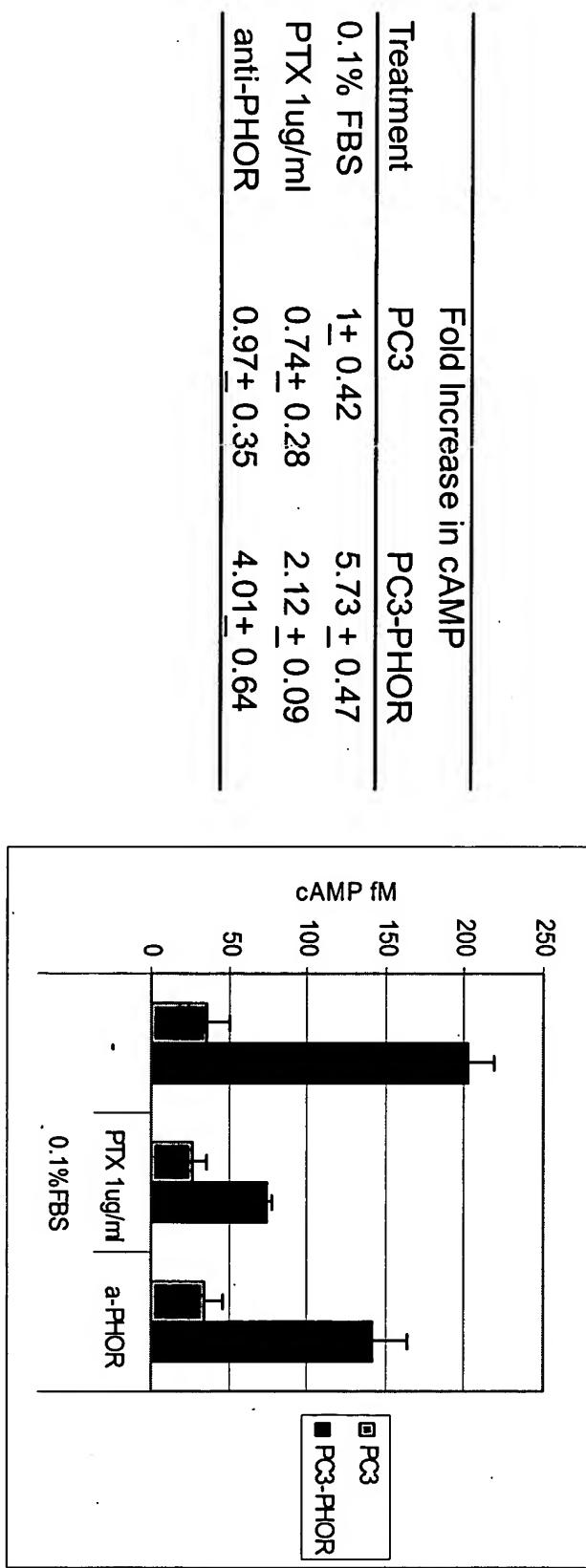
- Control NIH 3T3 and 3T3-101P3A11 cells were grown in the presence of absence 0.5 or 10% FBS. Proliferation was analyzed by Alamar blue after 48 hours.
- Expression of 101P3A11 induced a 6 fold increase in the proliferation of 3T3 cells grown in 0.5% FBS.

Figure 38: Inhibition of 101P3A11 Mediated ERK Phosphorylation by
101P3A11 Specific Antibodies



- Expression of 101P3A11 induced ERK phosphorylation in 293T cells.
- Anti-101P3A11 pAb inhibited ERK Phosphorylation in 293T-101P3A11 cells .

Figure 39: Anti-101P3A11 Ab Mediated cAMP Accumulation in PC3-
101P3A11 Cells



- Control PC3 cells and cells expressing 101P3A11 were treated with anti-101P3A11 pAb for 2 min and evaluated for intracellular cAMP content.
- The assay was performed in duplicate.

Figure 40A-40F

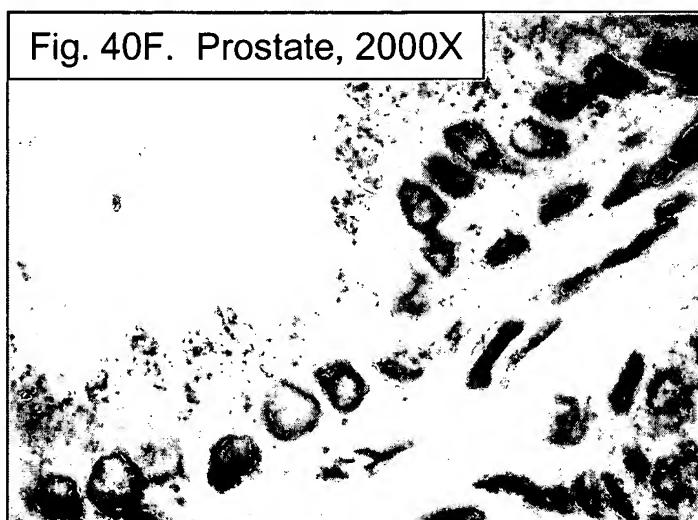
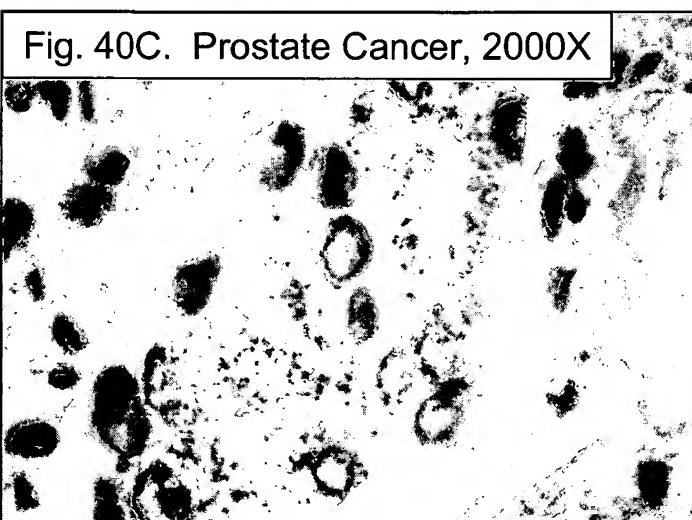
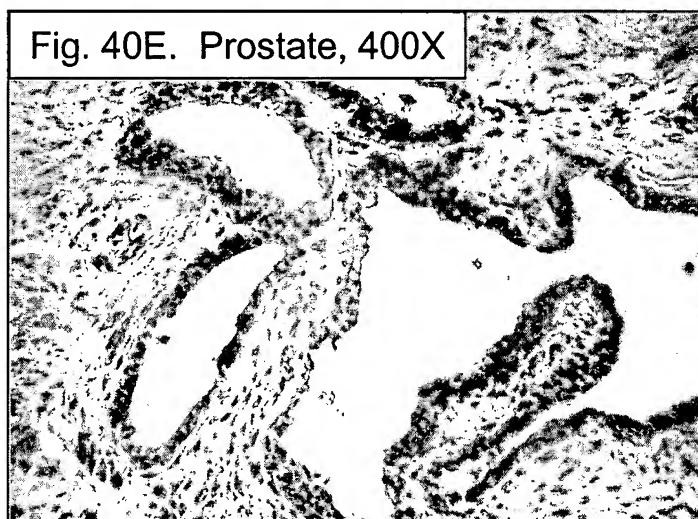
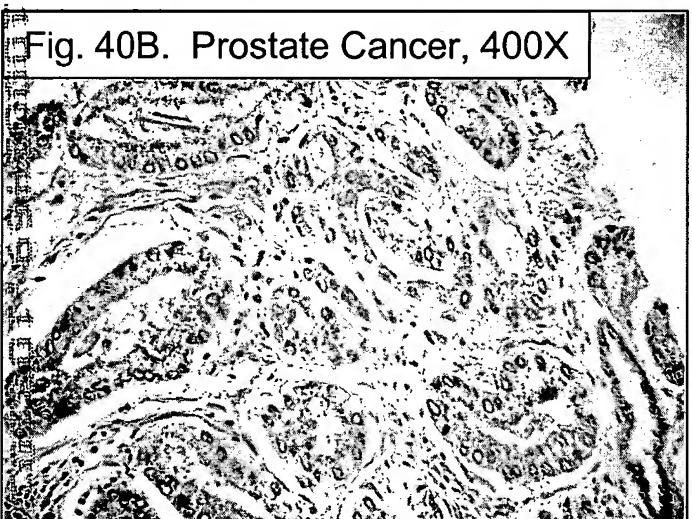
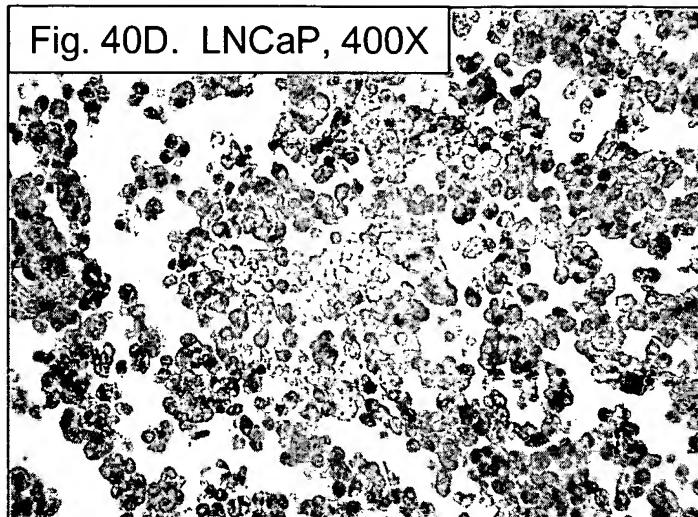
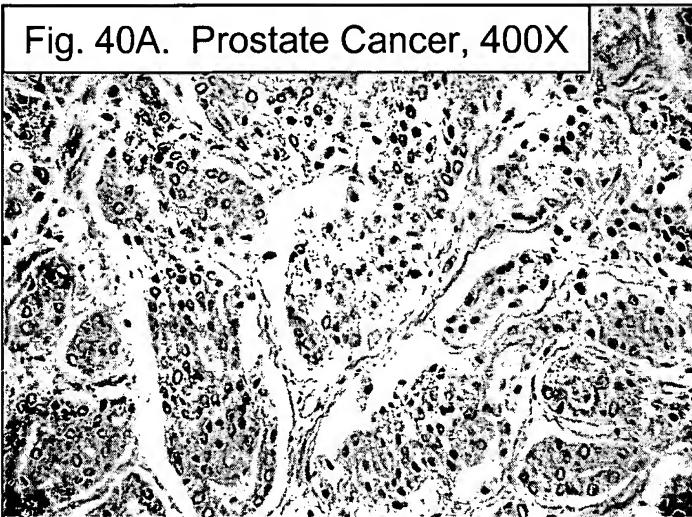


Fig.41A Prostate Cancer, 800X

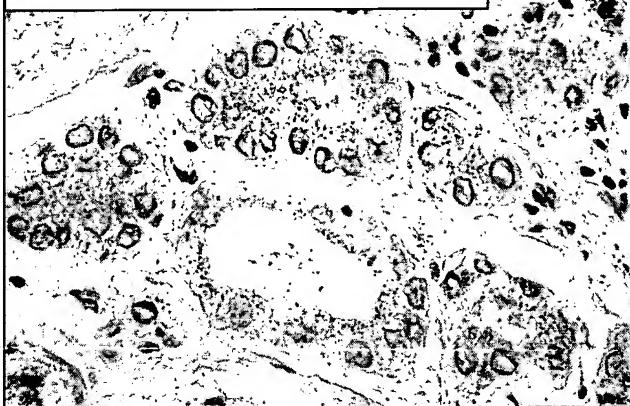


Fig.41B Bladder Cancer, 800X

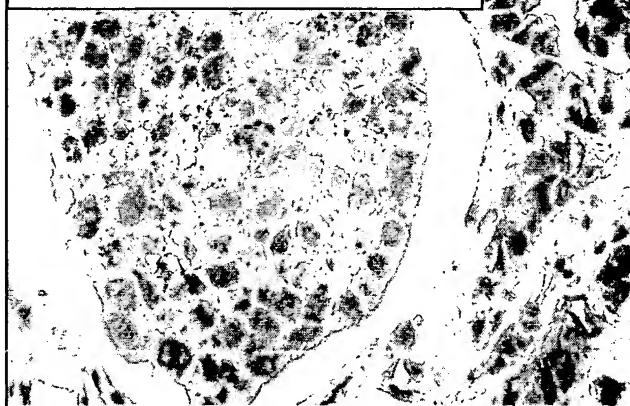


Fig.41C Kidney Cancer, 800X

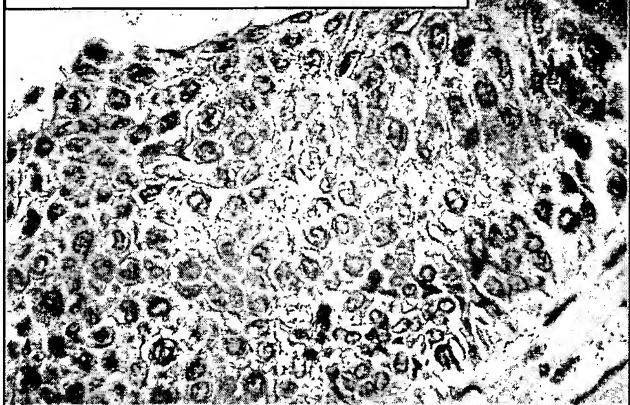


Fig.41D Colon Cancer, 800X

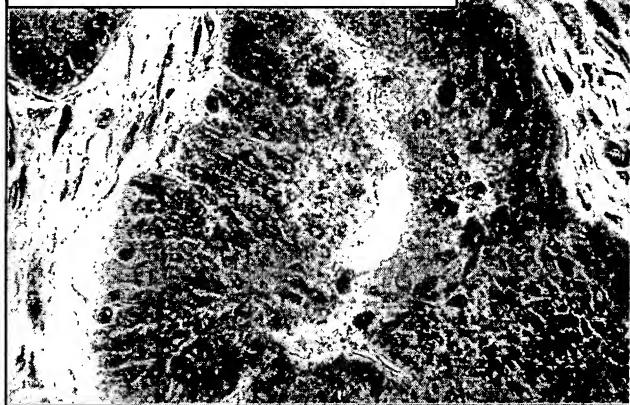


Fig.41E Lung Cancer, 800X

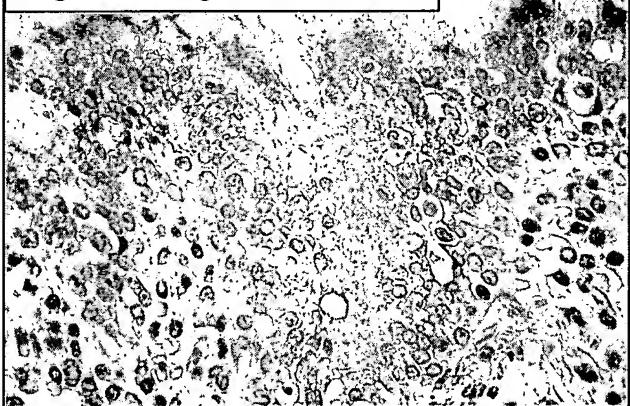
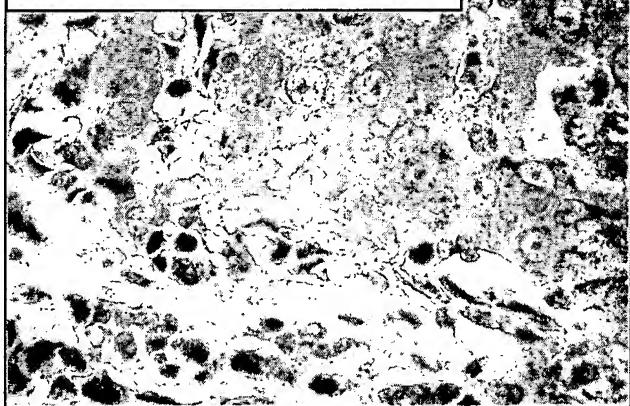


Fig.41F Breast Cancer, 800X



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Figure 42

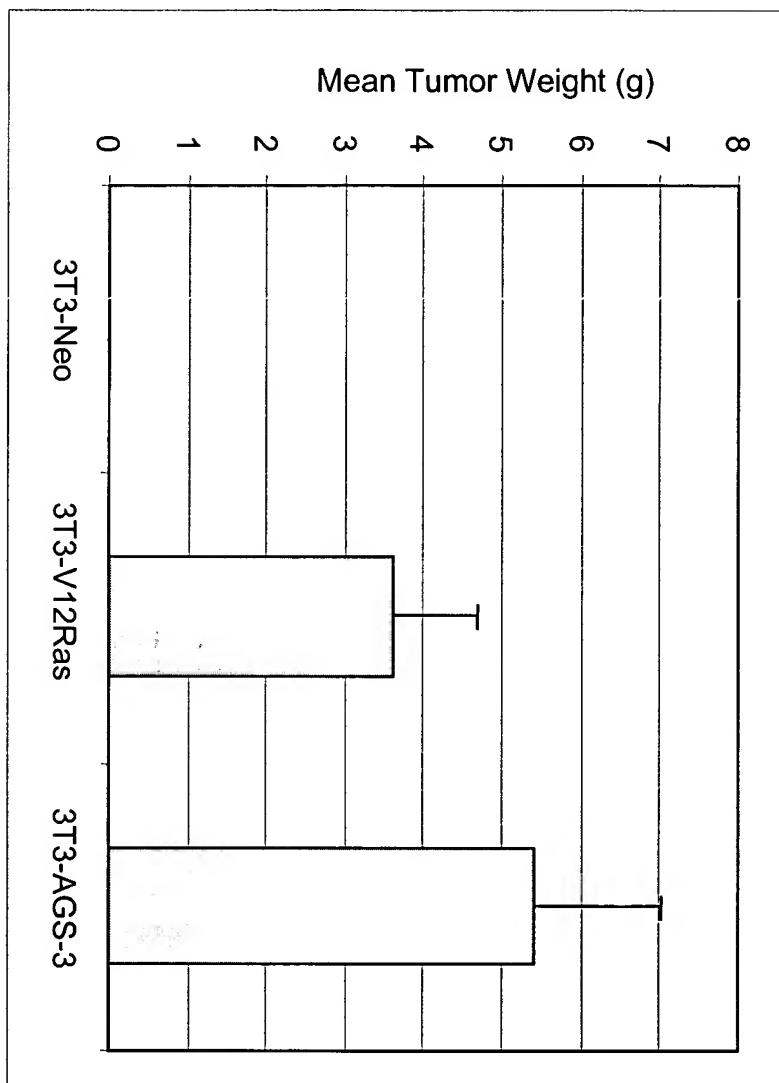


Figure 43

